10/525326

DT15 Rec'd PCT/PTO 22 FEB 2005

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Date: January 14, 2004

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10/525326
DT15 Rec'd PCT/PTO 22 FEB 2005

SPECIFICATION

AGENT FOR PREVENTION AND/OR TREATMENT OF ASTHMA

Technical Field

The present invention relates to an agent for prevention and/or treatment of asthma, which comprises a substance capable of suppressing the function involved in signal transduction of GPR4 as an active ingredient. The present invention further relates to an agent for prevention and/or treatment of asthma, which comprises a nitrogen-containing tricyclic compound or a quaternary ammonium salt thereof, or a pharmaceutically acceptable salt thereof as an active ingredient.

Background Art

Bronchial asthma is an inflammatory disease wherein bronchoconstriction and exacerbation of airway hyperreactivity are main characteristics. At present, daily control of asthma is considered to be fully conducted by a combination of an inhaled steroid, a bronchodilatory drug such as a β-stimulant or a xanthine-type drug and an anti-allergic drug represented by anti-leukotriene drugs. However, steroids which are mainly used for the treatment have side effects and there are patients who are resistant to steroids or are hardly cured by them, and therefore, there has been a demand for therapeutic agents having

a new mechanism and less side effects.

With regard to GPR4 which is a G-protein coupled-receptor protein (hereinafter, abbreviated as GPCR), it has been known to be highly expressed in a lung [*Genomics*, volume 30, pages 84- 88 (1995)]. In addition, it has been reported that GPR4 binds to a lipid such as sphingosyl phosphorylcholine (SPC) or lysophosphatidyl choline (LPC) to induce signals [*J. Biol. Chem.*, volume 276, pages 41325-41335 (2001)]. With regard to SPC, it has been reported to induce TNF-α production and ICAM-1 expression [*J. Invest. Dermatol.*, volume 112, pages 91-96 (1999)] and it has been suggested that it participates in allergic diseases such as skin diseases. With regard to LPC, it has been reported that it participates in migration of monocytes [*Cir. Res.*, volume 84, pages 52-59 (2000)], expression of adhesive molecules in endothelial cells [*J. Clin. Invest.*, volume 90, pages 1138-1144 (1992)], macrophage activation [*J. Immunol.*, volume 147, pages 273-280 (1991)], etc. and participates in inflammation. Further, with regard to LPC, there are other reports that it increases in plasma of patients suffering from asthma [*Clinic. Science*, volume 97, pages 595-601 (1999)] and that it increases in bronchoalveolar lavage fluid of allergic patients after antigen challenge [*J. Exp. Med.*, volume 183, pages 2235-2245 (1996)]. There is also a report that administration of choline which suppresses the production of LPC showed a therapeutic effect in patients suffering from

asthma [*Indian J. Chest Dis. Allied Sci.*, volume 39, pages 149-156 (1997)]. However, both SPC and LPC have been known to bind to OGR-1 [*Nat. Cell Biol.*, volume 2, pages 261-267 (2000)], G2A [*Science*, volume 293, pages 702-705 (2001)], etc. besides GPR4 and it has not been known whether such actions are mediated by GPR4.

Among the GPCRs, there has been known a GPCR called a constitutively activated GPCR, which transduces signals even in the absence of a ligand when it is excessively expressed in cells. A signal which is transduced in the absence of a ligand is called a constitutive activity. Among the constitutively activated GPCRs, there are those which are present in nature and those which are mutated by introducing a mutation such as substitution, deletion, etc. of amino acids [*Molecular Pharmacology*, volume 57, page 890 (2000); WO 98/46995]. An antagonist which suppresses the constitutive activity of GPCR is called an inverse agonist.

In the literatures [*Bulletin de la Société Chimique*, page 185 (1981) and *European Journal of Medicinal Chemistry*, volume 12, page 219 (1977)], compounds wherein R^1 represents morpholino, R^2, R^3 and R^4 represent hydrogen, a substituent corresponding to Y is morpholino, n is 1, and X represents $-(CH_2)_2-$ in the formula (I) which is described later are disclosed.

Disclosure of the Invention

An object of the present invention is:

1) to provide an agent for prevention and/or treatment of asthma, which comprises, as an active ingredient, a substance capable of suppressing the function involved in signal transduction of GPR4, and

2) to provide an agent for prevention and/or treatment of asthma, which comprises, as an active ingredient, a nitrogen-containing tricyclic compound or a quaternary ammonium salt thereof, or a pharmaceutically acceptable salt thereof.

The present invention relates to the following (1) to (8).

(1) An agent for prevention and/or treatment of asthma, which comprises, as an active ingredient, a substance capable of suppressing the function involved in signal transduction of a protein having the amino acid sequence represented by SEQ ID NO: 11.

(2) An agent for prevention and/or treatment of asthma, which comprises one of the following 1) to 4) as an active ingredient:

1) an oligonucleotide having a sequence complementary to that of oligonucleotide comprising continuous 5 to 60 nucleotides selected from the nucleotide sequence represented by SEQ ID NO: 12 or a derivative of said oligonucleotide,

2) an oligonucleotide having a sequence complementary

to that of oligonucleotide comprising continuous 5 to 60 nucleotides selected from the nucleotide sequence represented by SEQ ID NO: 14 or a derivative of said oligonucleotide,

3) an oligonucleotide having a sequence complementary to that of oligonucleotide comprising continuous 5 to 60 nucleotides selected from the nucleotide sequence represented by SEQ ID NO: 18 or a derivative of said oligonucleotide, and

4) an oligonucleotide comprising 5 to 60 nucleotides which hybridizes under stringent conditions with DNA having the nucleotide sequence represented by one member selected from SEQ ID NOs: 12, 14 and 18 and which is capable of suppressing the function involved in signal transduction of protein having the amino acid sequence represented by SEQ ID NO: 11 or a derivative of said oligonucleotide.

(3) An agent for prevention and/or treatment of asthma, which comprises one of the following 1) to 4) as an active ingredient:

1) an antibody which recognizes a protein having the amino acid sequence represented by SEQ ID NO: 11,

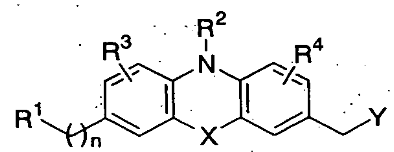
2) an antibody which recognizes a protein having the amino acid sequence represented by SEQ ID NO: 13,

3) an antibody which recognizes a protein having the amino acid sequence represented by SEQ ID NO: 17, and

4) an antibody, which recognizes a protein having the amino acid sequence in which one or more amino acid(s) is/are

deleted, substituted or added in the amino acid sequence represented by one member selected from SEQ ID NOs:11, 13 and 17 and which has the function involved in signal tranduction of a protein having the amino acid sequence represented by SEQ ID NO:11

(4) An agent for prevention and/or treatment of asthma, which comprises a nitrogen-containing tricyclic compound represented by the formula (I) or a quaternary ammonium salt thereof, or a pharmaceutically acceptable salt thereof;



(I)

[wherein R^1 represents a substituted or unsubstituted heterocyclic group, $-NR^5R^6$ (wherein R^5 and R^6 are the same or different and each represents hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aralkyl or substituted or unsubstituted heterocyclic alkyl, or R^5 and R^6 are combined together with the adjacent nitrogen atom to form a substituted or unsubstituted heterocyclic group), $-OR^7$ (wherein R^7 represents hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkanoyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted lower alkenyl,

substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted aralkyl or substituted or unsubstituted heterocyclic alkyl), $-SR^{7a}$ (wherein R^{7a} has the same meaning as the above R^7), $-CONR^{5a}R^{6a}$ (wherein R^{5a} and R^{6a} have the same meanings as the above R^5 and R^6, respectively), $-CO_2R^{7b}$ (wherein R^{7b} has the same meaning as the above R^7), $-N^+R^{5b}R^{6b}R^8$ (wherein R^{5b} and R^{6b} have the same meanings as the above R^5 and R^6, respectively, and R^8 represents lower alkyl, lower alkenyl or aralkyl), formyl, carboxy or cyano;

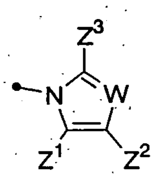
R^2 represents hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aralkyl or substituted or unsubstituted heterocyclic alkyl;

R^3 and R^4 are the same or different and each represents hydrogen, lower alkyl or halogen;

n represents 0 or 1;

X represents $-(CH_2)_2-$ or $-CH=CH-$; and

Y represents the formula (II);



(II)

(wherein W represents CH or a nitrogen atom;

Z^1 and Z^2 are the same or different and each represents hydrogen, substituted or unsubstituted lower alkyl, substituted or

unsubstituted cycloalkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted aralkyl or substituted or unsubstituted heterocyclic alkyl, or Z^1 and Z^2 are combined together with two carbon atoms being adjacent to each of them to form a substituted or unsubstituted aromatic ring or substituted or unsubstituted heterocycle; and

Z^3 represents hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, a substituted or unsubstituted heterocyclic group, substituted or unsubstituted aralkyl or substituted or unsubstituted heterocyclic alkyl)] as an active ingredient.

(5) The agent for prevention and/or treatment of asthma according to (4), wherein R^1 is $-NR^5R^6$ and R^5 and R^6 are combined together with the adjacent nitrogen atom to form a substituted or unsubstituted heterocyclic group.

(6) The agent for prevention and/or treatment of asthma according to (4) or (5), wherein R^2 is hydrogen.

(7) The agent for prevention and/or treatment of asthma according to any one of (4) to (6), wherein R^3 and R^4 are hydrogen.

(8) The agent for prevention and/or treatment of asthma according to any one of (4) to (7), wherein Z^1 and Z^2 are combined

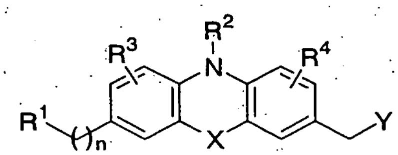
together with two carbon atoms being adjacent to each of them to form substituted or unsubstituted heterocycle.

(9) A method for prevention and/or treatment of asthma, which comprises administering an effective amount of the nitrogen-containing tricyclic compound or the quaternary ammonium salt thereof, or the pharmaceutically acceptable salt thereof described in any one of (4) to (8).

(10) Use of the nitrogen-containing tricyclic compound or the quaternary ammonium salt thereof, or the pharmaceutically acceptable salt thereof described in any one of (4) to (8) for the manufacture of an agent for prevention and/or treatment of asthma.

The present invention further relates to the following (11) to (23).

(11) A nitrogen-containing tricyclic compound represented by the formula (I) or a quaternary ammonium salt thereof, or a pharmaceutically acceptable salt thereof;



(I)

(wherein n, R^1, R^2, R^3, R^4, X and Y each have the same meanings as defined above, respectively).

(12) The nitrogen-containing tricyclic compound or the

quaternary ammonium salt thereof, or the pharmaceutically acceptable salt thereof according to (11), wherein R^1 is $-NR^5R^6$ and R^5 and R^6 are combined together with the adjacent nitrogen atom to form a substituted or unsubstituted heterocyclic group.

(13) The nitrogen-containing tricyclic compound or the quaternary ammonium salt thereof, or the pharmaceutically acceptable salt thereof according to (11) or (12), wherein R^2 is hydrogen.

(14) The nitrogen-containing tricyclic compound or the quaternary ammonium salt thereof, or the pharmaceutically acceptable salt thereof according to any one of (11) to (13), wherein R^3 and R^4 are hydrogen.

(15) The nitrogen-containing tricyclic compound or the quaternary ammonium salt thereof, or the pharmaceutically acceptable salt thereof according to any one of (11) to (14), wherein Z^1 and Z^2 are combined together with two carbon atoms being adjacent to each of them to form substituted or unsubstituted heterocycle.

(16) A pharmaceutical composition comprising the nitrogen-containing tricyclic compound or the quaternary ammonium salt thereof, or the pharmaceutically acceptable salt thereof according to any one of (11) to (15) as an active ingredient.

(17) A suppressor of the function involved in signal transduction of a protein having the amino acid sequence

represented by SEQ ID NO: 11, comprising the nitrogen-containing tricyclic compound or the quaternary ammonium salt thereof, or the pharmaceutically acceptable salt thereof according to any one of (11) to (15) as an active ingredient.

(18) A method for prevention and/or treatment of asthma, which comprises administering a therapeutically effective amount of a substance capable of suppressing the function involved in signal transduction of a protein comprising the amino acid sequence represented by SEQ ID NO: 11.

(19) A method for prevention and/or treatment of asthma, which comprises administering a therapeutically effective amount of an oligonucleotide or a derivative of said oligonucleotide which is any one of 1) to 4) described in (2).

(20) A method for prevention and/or treatment of asthma, which comprises administering a therapeutically effective amount of an antibody which is any one of 1) to 4) described in (3).

(21) Use of a substance capable of suppressing the function involved in signal transduction of a protein having the amino acid sequence represented by SEQ ID NO: 11 for the manufacture of an agent for prevention and/or treatment of asthma.

(22) Use of an oligonucleotide or a derivative of said oligonucleotide which is any one of 1) to 4) described in (2) for the manufacture of an agent for prevention and/or treatment of asthma.

(23) Use of an antibody which is any one of 1) to 4) described in (3) for the manufacture of an agent for prevention and/or treatment of asthma.

Thus, according to the present invention, there is provided a novel nitrogen-containing tricyclic compound or a quaternary ammonium salt thereof, or a pharmaceutically acceptable salt thereof described in (11) to (15) and there are provided a pharmaceutical composition comprising the same as an active ingredient and a suppressor of the function involved in signal transduction of a protein comprising the amino acid sequence represented by SEQ ID NO:11.

The present inventors have found a new finding that a substance capable of suppressing the function involved in signal transduction of GPR4 which belongs to GPCRs is effective for prevention and/or treatment of asthma and have achieved the present invention. The present inventors have searched substances capable of suppressing the constitutive activity of GPR4 which belongs to constitutively activated GPCRs and have found that a substance capable of suppressing the constitutive activity of GPR4 is effective for prevention and/or treatment of asthma.

Substances capable of suppressing the function involved in signal transduction of GPR4 include a substance capable of inhibiting or suppressing the expression of GPR4 itself, a substance capable of inhibiting the binding of a ligand to GPR4,

a substance capable of suppressing signal transduction caused by the binding of a ligand to GPR4 [such as changes (rise or fall) in intracellular cAMP concentration, changes (rise) in intracellular Ca^{2+} concentration and phosphorylation of mitogen-activated protein (MAP) kinase], a substance capable of suppressing signal transduction caused by a constitutive activity of GPR4 (such as an inverse agonist of GPR4), etc. There is no particular limitation for the structure for the above substances so far as they carry such functions, and substances having a known structure may be acceptable as well. Examples of GPR4 are a protein having the amino acid sequence represented by any one selected from SEQ ID NOs: 11, 13 and 17, a protein having the amino acid sequence in which one or more amino acid(s) is/are deleted, substituted or added in the amino acid sequence represented by any one selected from SEQ ID NOs: 11, 13 and 17 and which has the function involved in signal transduction of a protein having the amino acid sequence represented by SEQ ID NO: 11, etc.

The protein having an amino acid sequence wherein one or more amino acid(s) are/is deleted, substituted or added in the amino acid sequence represented by any one selected from SEQ ID NOs: 11, 13 and 17 and also having a function involved in the signal transduction of protein having the amino acid sequence represented by SEQ ID NO: 11 can be obtained, for example, by introducing a site-specific mutation into DNA encoding a

protein having an amino acid sequence represented by any one selected from SEQ ID NO: 11, 13 and 17 by site-directed mutagenesis described in the literatures [Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press (1989) (hereinafter, abbreviated as "Molecular Cloning, Second Edition"); Current Protocols in Molecular Biology, John Wiley & Sons (1987-1997) (hereinafter, abbreviated as "Current Protocols in Molecular Biology"); *Nucleic Acids Research*, 10, 6487 (1982); *Proc. Natl. Acad. Sci. USA*, 79, 6409 (1982); *Gene*, 34, 315 (1985); *Nucleic Acids Research*, 13, 4431 (1985); *Proc. Natl. Acad. Sci. USA*, 82, 488 (1985); etc.].

Although there is no particular limitation for the number(s) of amino acid(s) which is/are deleted, substituted or added, it/they is/are from 1 to several tens, preferably from 1 to 20, more preferably 1 to 10 and, still more preferably, 1 to 5.

The expression "one or more amino acid(s) are/is deletion, substitution or addition in the amino acid sequence represented by any one selected from SEQ ID NO: 11, 13 and 17" means that the amino acid sequence may contain deletion, substitution or addition of single or plural amino acid residue(s) at an arbitrary position therein. Deletion, substitution or addition may be simultaneously contained in one sequence and, the amino acid residue(s) to be deleted, substituted or added,

may be either natural or non-natural. Examples of natural amino acid residues are L-alanine, L-asparagine, L-aspartic acid, L-glutamine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-arginine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine and L-cysteine.

Preferred examples of amino acid residue(s) which is/are mutually able to be substituted are shown as hereunder. Amino acid residues belonging to the same group are able to be mutually substituted.

Group A: Leucine, isoleucine, norleucine, valine, norvaline, alanine, 2-aminobutanoic acid, methionine, O-methylserine, tert-butylglycine, tert-butylalanine and cyclohexylalanine;

Group B: Aspartic acid, glutamic acid, isoaspartic acid, isoglutamic acid, 2-aminoadipic acid and 2-aminosuberic acid;

Group C: Asparagine and glutamine;

Group D: Lysine, arginine, ornithine, 2,4-diaminobutanoic acid and 2,3-diaminopropionic acid;

Group E: Proline, 3-hydroxyproline and 4-hydroxyproline;

Group F: Serine, threonine and homoserine; and

Group G: Phenylalanine and tyrosine.

Also, in order that the protein having an amino acid sequence wherein one or more amino acid residue(s) is/are deleted, substituted or added in the amino acid sequence represented

by any one selected from SEQ ID NO: 11, 13 and 17 has a function involved in the signal transduction of the protein having an amino acid sequence represented by SEQ ID NO: 11, it is preferred that said amino acid sequence and the amino acid sequence represented by SEQ ID NO: 11 have a homology of at least 75%, usually not less than 80%, preferably not less than 90% or, more preferably, not less than 95%.

Homology of amino acid sequence and nucleotide sequence can be determined by using the algorithm BLAST by Karlin and Altschul [*Pro. Natl. Acad. Sci. USA*, 90, 5873 (1993)] or by FASTA [*Methods Enzymol.*, 183, 63 (1990)]. On the basis of the algorithm BLAST, programs called BLASTN (database for nucleotide sequence vs. nucleotide sequence) and BLASTX (database for nucleotide sequence vs. amino acid sequence) have been developed [*J. Mol. Biol.*, 215, 403 (1990)]. When a nucleotide sequence is analyzed by BLASTN based upon BLAST, for example, parameters can be set to score = 100 and wordlength = 12. When an amino acid sequence is analyzed by BLASTX based upon BLAST, for example, parameters can be set to score = 50 and wordlength = 3. When BLAST and Gapped BLAST programs are used, a default parameter for each program can be used. [With regard to Gapped BLAST, refer to the literature (*Nuc. Acids Res.*, 25, 3389-3402 (1997)).] Specific means for those analytical methods are known (refer to http://www.ncbi.nlm.nih.gov).

Examples of a substance capable of inhibiting or suppressing the expression of GPR4 itself are an oligonucleotide (hereinafter, referred to as "antisense oligonucleotide") having a complementary sequence of oligonucleotide comprising continuous 15 to 60 nucleotides selected from the nucleotide sequence represented by any one selected from SEQ ID NO: 12, 14 and 18; an oligonucleotide which hybridizes under stringent conditions with DNA having the nucleotide sequence represented by any one selected from SEQ ID NO: 12, 14 and 18 and suppresses the function involved in signal transduction of protein having the amino acid sequencerepresented by SEQ ID NO: 11; a derivative of the oligonucleotide as such (hereinafter, referred to as "oligonucleotide derivative"); etc.

The antisense oligonucleotide mentioned in the above is not particular limited so far as it is an antisense oligonucleotide having a sequence complementary to an oligonucleotide comprising continuous 15 to 60 nucleotides selected from the nucleotide sequences represented by any one selected from SEQ ID NO: 12, 14 and 18 although preferred one is an antisense oligonucleotide having 17 to 60 nucleotides, more preferably 20 to 60 nucleotides and, still more preferably, 30 to 60 nucleotides. Particularly preferred one is an antisense oligonucleotide having a complementary sequence of translation initiation region of the above-mentioned oligonucleotide. Said antisense oligonucleotide can be

prepared by a conventional method such as by using a DNA synthesizer on the basis of information concerning a nucleotide sequence for a nucleotide sequence represented by any one selected from SEQ ID NOs: 12, 14 and 18 or for a nucleotide sequence of fragment thereof.

Examples of the oligonucleotide derivative are an oligonucleotide derivative wherein a phosphoric acid diester bond in an oligonucleotide is converted into a phosphorothioate bond; an oligonucleotide derivative wherein a phosphoric acid diester bond in an oligonucleotide is converted to an N3'-P5' phosphoamidate bond; an oligonucleotide derivative wherein a bond of phosphoric acid diester with ribose in an oligonucleotide is converted to a peptide-nucleic acid bond; an oligonucleotide derivative wherein uracil in an oligonucleotide is substituted with C-5 propynyluracil; an oligonucleotide derivative wherein uracil in an oligonucleotide is substituted with C-5 thiazolyluracil; an oligonucleotide derivative wherein cytosine in an oligonucleotide is substituted with C-5 propynylcytosine; an oligonucleotide derivative wherein cytosine in an oligonucleotide is substituted with a phenoxazine-modified cytosine; an oligonucleotide derivative wherein ribose in an oligonucleotide is substituted with 2'-O-propylribose; and an oligonucleotide derivative wherein ribose in an oligonucleotide is substituted with 2'-methoxyethoxyribose [*Saibo Kogaku*, 16, 1463 (1997)].

The expression of GPR4 itself can be inhibited or suppressed by using the above-mentioned antisense oligonucleotide or oligonucleotide derivative in accordance with an antisense RNA/DNA technology [*Bioscience and Industry*, 50, 322 (1992); *Kagaku*, 46, 681 (1991); *Biotechnology*, 9, 358 (1992); *Trends in Biotechnology*, 10, 87 (1992); *Trends in Biotechnology*, 10, 152 (1992); *Saibo Kogaku*, 16, 1463 (1997)], a triple helix technology [*Trends in Biotechnology*, 10, 132 (1992)], a ribozyme technology [*Current Opinion in Chemical Biology*, 3, 274 (1999); *FEMS Microbiology Reviews*, 23, 257 (1999); *Frontiers in Bioscience*, 4, D497 (1999); *Chemistry & Biology*, 6, R33 (1999); *Nucleic Acids Research*, 26, 5237 (1998); *Trends in Biotechnology*, 16, 438 (1998)] or a decoy DNA method [*Nippon Rinsho - Japanese Journal of Clinical Medicine*, 56, 563 (1998); *Circulation Research*, 82, 1023 (1998); *Experimental Nephrology*, 5, 429 (1997); *Nippon Rinsho - Japanese Journal of Clinical Medicine*, 54, 2583 (1996)].

The oligonucleotide which hybridizes under stringent conditions to DNA having the nucleotide sequence represented by any one selected from SEQ ID NOs: 12, 14 and 18 is a DNA which is obtained by colony hybridization, plaque hybridization, Southern blot hybridization, etc. using a part of or whole DNA having the nucleotide sequence represented by any one selected from SEQ ID NOs: 12, 14 and 18 as a probe. To be more specific, the DNA includes a DNA which can be identified by carrying out

hybridization at 65°C in the presence of 0.7 to 1.0 mol/l of sodium chloride using a filter on which a DNA prepared from colonies or plaque is immobilized, and then washing the filter under the condition of 65°C using an SSC solution of 0.1- to 2-fold concentration (composition of an SSC solution of a 1-fold concentration comprises 150 mmol/l of sodium chloride and 15 mmol/l of sodium citrate). Hybridization can be carried out according to a method mentioned, for example, in Molecular Cloning, Second Edition; Current Protocols in Molecular Biology; DNA Cloning 1: Core Techniques, A Practical Approach, Second Edition, Oxford University (1995). The oligonucleotide which is hybridizable includes a DNA having at least 75% homology preferably not less than 80% homology, more preferably not less than 95% homology to DNA having a complementary sequence of DNA having the nucleotide sequence represented by any one selected from SEQ ID Nos: 12, 14 and 18 when calculated by, for example, the above-mentioned BLAST or FASTA. DNA, RNA and the like can be used as the oligonucleotide, and DNA can be used preferably.

A formulation prepared according to the following conventional method from the antisense oligonucleotide or the derivative of said antisense oligonucleotide described in the above, or the oligonucleotide or the derivative of said oligonucleotide, wherein the oligonucleotide hybridizes under stringent conditions to DNA having the nucleotide sequence

represented by any of selected from SEQ ID NOs: 12, 14 and 18, either solely or after inserting into a vector for gene therapy such as a retrovirus vector, an adenovirus vector or an adenovirus associated virus vector or the like, also can be used as an agent for prevention and/or treatment of asthma.

When the vector for gene therapy is used as said agent for prevention and/or treatment, it is able to be manufactured by compounding said vector for gene therapy with a carrier used for gene therapy agent [*Nature Genet.*, 8, 42 (1994)].

With regard to the above carrier, any carrier may be used so far as it is a carrier which is commonly used for injection preparations and its examples are distilled water, a salt solution such as sodium chloride or a mixture of sodium chloride or inorganic salt, a solution of saccharide such as mannitol, lactose, dextran and glucose, a solution of amino acid such as glycine and arginine and a mixed solution of organic acid solution or a salt solution with glucose solution. It is also possible that, in accordance with the common method, an excipient such as osmotic pressure adjusting agent, pH adjusting agent, plant oil such as sesame oil or soybean oil, lecithin or surfactant such as nonionic surfactant is added for the carrier whereupon an injection solution is prepared as solution, suspension or dispersion. It is further possible that such an injection solution is prepared in a form of being dissolved immediately before use after an operation such as pulverization

and freeze-drying.

Said agent for prevention and/or treatment may be used as it is when the agent is liquid or, when the agent is solid, it may be used after dissolving, immediately before use, in the above-mentioned carrier which is subjected to a sterilization treatment if necessary.

An example of the method for administration is a local administration so that it is able to be absorbed with the site of the patient to be treated. It is also possible to transport the DNA to the aimed site for the treatment by means of a non-viral gene transfection.

Examples of the non-viral gene transfection method are a calcium phosphate coprecipitation method [*Virology*, 52, 456-467 (1973); *Science*, 209, 1414-1422 (1980)], a microinjection method [*Proc. Natl. Acad. Sci. USA*, 77, 5399-5403 (1980); *Proc. Natl. Acad. Sci. USA*, 77, 7380-7384 (1980); *Cell*, 27, 223-231 (1981); *Nature*, 294, 92-94 (1981)]; a membrane fusion-intervening transfection method using liposome [*Proc. Natl. Acad. Sci. USA*, 84, 7413-7417 (1987); *Biochemistry*, 28, 9508-9514 (1989); *J. Biol. Chem.*, 264, 12126-12129 (1989); *Hum. Gene Ther.*, 3, 267-275 (1992); *Science*, 249, 1285-1288 (1990); *Circulation*, 83, 2007-2011 (1992)], a direct DNA-incorporation or receptor-mediated DNA transfection method [*Science*, 247, 1465-1468 (1990); *J. Biol. Chem.*, 266, 14338-14342 (1991); *Proc. Natl. Acad. Sci. USA*, 87, 3655-3659 (1991); *J. Biol. Chem.* 264,

16985-16987 (1989); *BioTechniques*, 11, 474-485 (1991); *Proc. Natl. Acad. Sci. USA*, 87, 3410-3414 (1990); *Proc. Natl. Acad. Sci. USA*, 88, 4255-4259 (1991); *Proc. Natl. Acad. Sci. USA*, 87, 4033-4037 (1990); *Proc. Natl. Acad. Sci. USA*, 88, 8850-8854 (1991); *Hum. Gene Ther.* 3, 147-154 (1991)], etc.

The substance which inhibits a binding of a ligand to GPR4 includes an antibody which recognizes GPR4, a compound which has an antagonistic action to GPR4, or the like.

With regard to the above-mentioned antibody, any antibody may be used so far as it is an antibody which recognizes GPR4 although an antibody which specifically recognizes GPR4 is preferred. Said antibody may be either a polyclonal antibody or a monoclonal antibody. With regard to such an antibody, an example is a neutraizing antibody which recognizes GPR4. A chimera antibody of a human type, a humanized antibody, and the like also can be used as an antibody of the present invention.

The above-mentioned antibody can, for example, be prepared according to the following methods.

(1) Preparation of a polyclonal antibody

A polyclonal antibody can be prepared in such a manner that purified sample of GPR4 or a partial fragment polypeptide thereof or a peptide having a part of amino acid sequence of GPR4 is used as an antigen and administered to an animal.

With regard to the animal to which administration is conducted, it is possible to use rabbit, goat, rat, mouse,

hamster, etc.

The amount of said antigen to be adiministered is preferred to be 50 to 100 μg per animal.

When a peptide is used, it is preferred to use a product wherein a peptide is subjected to a covalent bond to a carrier protein such as keyhole limpet hemocyanin, bovine thyroglobulin, or the like. A peptide used as an antigen may be synthesized by a peptide synthesizer.

Administration of said antigen is conducted for three to ten times every one or two week(s) after the first administration. After each administration, blood is collected from venous plexus of fundus of the eye on the third to the seventh day and it is confirmed by means of an enzyme-linked immunosorbent assay [ELISA Method: published by Igaku Shoin (1976); Antibodies - A Laboratory Manual, Cold Spring Harbor Laboratory (1988)] or the like that said serum reacts with an antigen used for immunization.

Serum is obtained from non-human mammals wherein the serum thereof shows a sufficient antibody value to the antigen used for immunization and said serum is separated and purified whereupon a polyclonal antibody is able to be prepared.

With regard to a method for the separation and the purification, it is possible to conduct a treatment by centrifugal separation, salting-out using 40-50% saturated ammonium sulfate, precipitation with caprylic acid [Antibodies

- A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)], chromatography using DEAE-Sepharose column, anion exchange column, protein A or G column, gel filtration column or the like, etc. either solely or in combination.

(2) Preparation of monoclonal antibody

(a) Preparation of antibody-producing cells

To the pure sample of GPR4 used for immunization or the partial fragment polypeptide thereof or to the peptide having an amino acid sequence of a part of GPR4 is used a rat where its serum shows a sufficient antibody value as a supplying source of antibody-producing cells.

After the third to the seventh day from the final administration of an antigen substance to the rat showing the antibody value, its spleen is excised.

The spleen is finely cut in an MEM medium (manufactured by Nissui Pharmaceutical), loosened using a forceps and centrifuged at 1,200 rpm for 5 minutes and a supernatant liquid is discarded.

Spleen cells of the resulting precipitate fraction is treated with Tris-ammonium chloride buffer (pH 7.65) for 1 to 2 minute(s) to remove erythrocytes and washed with an MEM medium for three times and the resulting spleen cells are used as antibody-producing cells.

(b) Preparation of myeloma cells

Established cell line prepared from mouse or rat is used

as myeloma cell. For example, it is possible to use 8-azaguanine resistant mouse (derived from BALB/c) myeloma cell line P3-X63Ag8-U1 (hereinafter, abbreviated as P3-U1) [*Curr. Topics Microbiol. Immunol.*, 81, 1 (1978); *Eur. J. Immunol.*, 6, 511 (1976)], SP2/0-Ag14 (SP-2) [*Nature*, 276, 269 (1978)], P3-X63-Ag8653 (653) [*J. Immunol.*, 123, 1548 (1979)], P3-X63-Ag8 (X63) [*Nature*, 256, 495 (1975)], etc. Those cell lines are passaged using an 8-azaguanine medium [8-azaguanine (15 μg/ml) is further added to a medium (hereinafter, referred to as "normal medium") wherein glutamine (1.5 mmol/l), 2-mercaptoethanol (5×10^{-5} mol/l), gentamicin (10 μg/ml) and fetal bovine serum (FCS) (manufactured by CSL; 10%) are added to an RPMI-1640 medium]. Thus incubation is conducted on a normal medium before 3 to 4 days of cell fusion and not less than 2×10^7 of said cells are used for the fusion.

(c) Preparation of hybridoma

The antibody-producing cells prepared in (a) and the myeloma cells prepared in (b) are well washed with an MEM medium or a PBS (1.83 g of disodium phosphate, 0.21 g of monopotassium phosphate, 7.65 g of sodium chloride and 1 liter of distilled water; pH 7.2) and mixed so as to make the ratio of (antibody-producing cells) : (myeloma cells) = 5 to 10 : 1, the mixture is centrifuged at 1,200 rpm for 5 minutes and a supernatant liquid is discarded.

Cell group of the resulting precipitate fraction is well

loosened, 0.2 to 1 ml of a solution wherein 2 g of polyethylene glycol (PEG-1000), 2 ml of MEM and 0.7 ml of dimethyl sulfoxide (DMSO) are mixed is added to said cell group per 10^8 antibody-producing cells with stirring at 37°C and then 1 to 2 ml of an MEM medium is added for several times every 1 to 2 minute(s).

After addition, an MEM medium is added so as to prepare a solution in an amount of 50 ml. Said prepared solution is centrifuged at 900 rpm for 5 minutes and a supernatant liquid is discarded. Cells of the resulting precipitate fraction are gently loosened and gently suspended in 100 ml of an HAT medium [a medium where hypoxanthine (10^{-4} mol/l), thymidine (1.5×10^{-5} mol/l) and aminopterin (4×10^{-7} mol/l) are added to a normal medium] by means of suction and spouting using a measuring pipette.

Said suspension is dispensed in a 96-well incubation plate in an amount of 100 µl per well and is incubated in a 5% CO_2 incubator at 37°C for 7 to 14 days.

After the incubation, a part of a supernatant liquid thereof is taken out and a hybridoma which specifically reacts with the partial fragment polypeptide of the polypeptide of the present invention is selected by an enzymatic immunoassay mentioned, for example, in Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Chapter 14 (1988).

The following methods may be listed as specific examples

of the enzymatic immunoassay.

In immunization, GPR4 used as antigen or a pure sample of a partial fragment polypeptide thereof or a peptide having a partial amino acid sequence of GPR4 is coated on an appropriate plate, subjected to a reaction using supernatant liquid of incubated hybridoma or the pure antibody prepared in (d) which is mentioned later as the first antibody, then subjected to a reaction using anti-rat or anti-mouse immunoglobulin labeled with biotin, enzyme, chemoluminescent substance or radiation compound as the second antibody and subjected to a reaction in accordance with a labeled substance and that which specifically reacts with a polypeptide used as an antigen is selected as a hybridoma which produces a monoclonal antibody to be used in the present invention.

Cloning by a limiting dilution method using said hybridoma is repeated twice [in the first one, an HT medium (a medium wherein aminopterin is removed from an HAT medium) is used and, in the second one, a normal medium is used] and that which shows a strong antibody value in a stable manner is selected as a hybridoma strain producing a monoclonal antibody to be used in the present invention.

(d) Preparation of monoclonal antibody

The hybridoma cells (5 to 20×10^6 cells/animal) producing monoclonal antibody to be used in the present invention which are prepared in (c) are intraperitoneally injected to mouse

or nude mouse of 8 to 10 weeks age treated with pristane [0.5 ml of pristane (2,6,10,14-tetramethylpentadecane) is intraperitoneally injected followed by breeding for two weeks]. Hybridoma becomes ascites tumor within 10 to 21 days.

Ascites is collected from said mouse becoming ascites tumor and centrifuged at 3,000 rpm for 5 minutes to remove solid.

From the resulting supernatant liquid, monoclonal antibody is able to be purified and prepared by the method similar to that used for polyclonal antibody.

Determination of subclass of the antibody is carried out using a mouse monoclonal antibody typing kit or a rat monoclonal antibody typing kit. Amount of the polypeptide is calculated by a Lowry method or from the absorbance at 280 nm.

An agent for prevention and/or treatment of asthma comprising the above-mentioned antibody capable of recognizing GPR4 is able to be prepared as follows.

With regard to a medicament comprising said antibody as an active ingredient, although it is possible that said active ingredient is administered solely, it is usually preferred to provide as a pharmaceutical preparation by mixing of said active ingredient with one or more pharmaceutically acceptable carrier(s) followed by manufacturing by any method which is well known in the technical field of pharmaceutical preparation sciences. Preferably, an aseptic solution being dissolved in an aqueous carrier such as water or aqueous solution of sodium

chloride, glycine, glucose, human albumin, etc. is used. It is also possible to add a pharmaceutically acceptable additive such as buffering or isotonizing agent for making the pharmaceutical preparation solution nearer the physiological condition such as sodium acetate, sodium chloride, sodium lactate, potassium chloride and sodium citrate. It is further possible to preserve by freeze-drying and to dissolve in an appropriate solvent in actual use.

With regard to the administering route, it is desirable to use that which is most effective for the therapy and its examples are oral administration and parenteral administration such as intravenous injection. Examples of the dosage form are tablets, injections, or the like.

With regard to the preparation suitable for oral administration, tablets may be exemplified. They are able to be manufactured using additives including excipient such as lactose and mannitol, disintegrating agent such as starch, lubricant such as magnesium stearate, binder such as hydroxypropyl cellulose, surfactant such as fatty acid ester, plasticizer such as glycerol, etc.

With regard to the preparation suitable for parenteral administration, injections may be exemplified. They may be prepared, for example, using a carrier comprising a salt solution, a glucose solution or a mixture thereof. It is also possible to use the components exemplified as additives in the oral

preparation even in the case of parenteral preparation.

Dose and administering frequency may vary depending upon aimed therapeutic effect, administering method, period of treatment, age, body weight, etc. and, usually, they are from 10 μg/kg to 8 mg/kg per day for adults.

A substance which is capable of suppressing the function involved in signal transduction resulted from a constitutive activity of GPR4 is also able to be prepared by investigating the substances which are able to suppress the signal transduction resulted by said constitutive activity.

An example of the compounds having an antagonistic action to GPR4 is a compound represented by the formula (I). Hereinafter, a compound represented by the formula (I) is referred to as compound (I). That is also applied to compounds having other formula numbers.

In definitions of the groups in compound (I), the following exemplification is listed.

(i) With regard to the lower alkyl and the lower alkyl moiety in the lower alkanoyl, linear or branched alkyl having 1 to 10 carbon(s) may be exemplified and specific examples thereof are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, neopentyl, hexyl, heptyl, octyl, isooctyl, nonyl and decyl.

(ii) With regard to the cycloalkyl, cycloalkyl having 3 to 8 carbons may be exemplified and specific examples thereof

are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

(iii) With regard to the lower alkenyl, linear, branched or cyclic alkenyl having 2 to 8 carbons may be exemplified and specific examples thereof are vinyl, allyl, 1-propenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, cyclohexenyl and 2,6-octadienyl.

(iv) With regard to the lower alkynyl, linear or branched alkynyl having 2 to 8 carbons may be exemplified and specific examples thereof are ethynyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl and octynyl.

(v) The halogen means each of fluorine, chlorine, bromine and iodine atoms.

(vi) With regard to the aryl and a group wherein one hydrogen atom is removed from the aromatic ring formed together with two carbon atoms being adjacent to each, monocyclic, bicyclic or tricyclic aryl having 6 to 14 carbons may be exemplified and specific examples thereof are phenyl, naphthyl, indenyl and anthranyl.

(vii) The alkylene moiety of the aralkyl and the heterocyclic alkyl has the same meaning as that where one hydrogen atom is removed from the definition for the above lower alkyl (i).

(viii) With regard to the aryl moiety of the aralkyl, a bicyclic fused ring group wherein the above aryl is fused

to cycloalkyl may be exemplified in addition to the groups exemplified in the definition for the above aryl (vi) and specific examples thereof are indanyl, 1,2,3,4-tetrahydronaphthyl and 6,7,8,9-tetrahydro-5H-benzocycloheptyl.

(ix) With regard to the heterocyclic group, the heterocyclic moiety of the heterocyclic alkyl and a group wherein one hydrogen atom is removed from the heterocyclic ring formed together with two carbon atoms being adjacent to each, a five- or six-membered monocyclic heterocyclic group containing at least one atom selected from a nitrogen atom, an oxygen atom and a sulfur atom and a fused ring heterocyclic group which is bicyclic or tricyclic where three- to eight-membered rings are fused and which contains at least one atom selected from a nitrogen atom, an oxygen atom and a sulfur atom may be exemplified and specific examples thereof are pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, benzimidazolyl, 2-oxobenzimidazolyl, benzotriazolyl, benzofuryl, benzothienyl, purinyl, benzoxazolyl, benzothiazolyl, benzodioxolyl, indazolyl, indolyl, isoindolyl, quinolyl, isoquinolyl, phthalazinyl, naphthylidinyl, quinoxalinyl, pyrrolyl, pyrazolyl, quinazolinyl, cinnolinyl, triazolyl, tetrazolyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, thienyl, furyl, pyrrolidinyl, 2,5-dioxopyrrolidinyl, thiazolidinyl, oxazolidinyl,

piperidyl, piperidino, piperazinyl, homopiperazinyl, homopiperidyl, homopiperidino, morpholinyl, morpholino, thiomorpholinyl, thiomorpholino, pyranyl, tetrahydropyridyl, tetrahydropyranyl, tetrahydrofuranyl, tetrahydroquinolyl, tetrahydroisoquinolyl, octahydroquinolyl and indolinyl.

(x) With regard to the heterocyclic group formed together with the adjacent nitrogen atom, a five- to six-membered monocyclic heterocyclic group containing at least one nitrogen atom (said monocyclic heterocyclic group may contain other nitrogen atom, oxygen atom or sulfur atom) and a fused ring heterocyclic group which is bicyclic or tricyclic where three- to eight-membered rings are fused and which contains at least one nitrogen atom (said fused ring heterocyclic group may contain other nitrogen atom, oxygen atom or sulfur atom) may be exemplified and specific examples thereof are pyridyl, tetrahydropyridyl, indolinyl, isoindolinyl, pyrrolidinyl, thiazolinyl, oxazolidinyl, piperidino, homopiperidino, piperazinyl, homopiperazinyl, morpholino, thiomorpholino, perhydroazepinyl, perhydroazocinyl, tetrahydroquinolyl, tetrahydroisoquinolyl, octahydroquinolyl, benzimidazolyl, indazolyl, indolyl, isoindolyl, purinyl, dihydroindolyl, pyrrolyl, dihydropyrrolyl, pyrazolyl, triazolyl, tetrazolyl and imidazolyl.

(xi) With regard to the substituent in the substituted lower alkyl and the substituted lower alkanoyl, the number of

the substituent(s), which may be the same or different, is 1 to 3 and examples thereof are the following. Thus, cycloalkyl, lower alkanoyl, lower alkoxy, aryloxy, substituted aryloxy [with regard to the substituent in said substituted aryloxy, the number of the substituent(s), which may be the same or different, is 1 to 3 and examples thereof are lower alkyl, lower alkoxy, lower alkoxycarbonyl, halogen, cyano, nitro, hydroxy, carboxy and amino; here, the lower alkyl has the same meaning as the above-mentioned lower alkyl (i), the halogen has the same meaning as the above-mentioned halogen (v) and the lower alkyl moiety in the lower alkoxy and the lower alkoxycarbonyl has the same meaning as the above-mentioned lower alkyl (i)], aralkyloxy, substituted aralkyloxy [with regard to the substituent in said substituted aralkyloxy, the number of the substituent(s), which may be the same or different, is 1 to 3 and examples thereof are lower alkyl, lower alkoxy, lower alkoxycarbonyl, halogen, cyano, nitro, hydroxy, carboxy and amino; here, the lower alkyl has the same meaning as the above-mentioned lower alkyl (i), the halogen has the same meaning as the above-mentioned halogen (v) and the lower alkyl moiety in the lower alkoxy and the lower alkoxycarbonyl has the same meaning as the above-mentioned lower alkyl (i)], lower alkanoyoxy, lower alkoxycarbonyl, halogen, cyano, nitro, hydroxy, carboxy, amino, lower alkylthio, substituted lower alkyl [with regard to the substituent in said substituted lower

alkyl, the number of the substituent(s), which may be the same or different, is 1 to 3 and examples thereof are hydroxy and halogen], the substituted lower alkanoyl [with regard to the substituent in the substituted lower alkanoyl, the number of the substituent(s), which may be the same or different, is 1 to 3 and an example thereof is halogen], mono- or di-lower alkylamino, lower alkylsulfonyl, lower alkylsulfinyl, lower alkoxycarbonylamino, lower alkanoylamino, mono- or di-lower alkylaminocarbonyl, mono- or di-lower alkylaminocarbonyloxy, a heterocyclic group and the like are exemplified.

The aryl moiety in the aryloxy and the aralkyloxy, the cycloalkyl, the halogen, the heterocyclic group and the lower alkyl moiety in the lower alkanoyl, the lower alkoxy, the lower alkanoyloxy, the lower alkoxycarbonyl, the lower alkylthio, the lower alkylsulfonyl, the lower alkylsulfinyl, the lower alkoxycarbonylamino and the lower alkanoylamino shown here have the same meanings as the above-mentioned aryl (vi), cycloalkyl (ii), halogen (v), heterocyclic group (ix) and lower alkyl (i), respectively. The alkylene moiety in the aralkyloxy has the same meaning as that where one hydrogen atom is removed from the above-mentioned lower alkyl (i).

The lower alkyl moiety of the mono- or di-lower alkylamino, the mono- or di-lower alkylaminocarbonyl and the mono- or di-lower alkylaminocarbonyloxy each has the same meaning as the above-mentioned lower alkyl (i). Two lower alkyl moieties

in the di-lower alkylamino, the di-lower alkylaminocarbonyl and the di-lower alkylaminocarbonyloxy each may be the same or different.

(xii) With regard to the substituent in the substituted aryl, the substituted aralkyl, the substituted cycloalkyl, the substituted lower alkenyl, the substituted lower alkynyl, the substituted heterocyclic group, the substituted heterocyclic alkyl, the substituted heterocyclic group formed together with the adjacent nitrogen atom, the substituted aromatic ring formed together with two carbon atoms being adjacent to each and the substituted heterocycle formed together with two carbon atoms being adjacent to each, in addition to the above-mentioned groups exemplified in the definition for the substituent (xi) in the substituted lower alkyl are lower alkyl, aryl, substituted aryl, aralkyl, substituted aralkyl, a heterocyclic group, a substituted heterocyclic group, heterocyclic alkyl, substituted heterocyclic alkyl and the like are exemplified. Further, with regard to the substituent in the substituted aryl and the substituted heterocyclic group formed together with the adjacent nitrogen atom, it may be lower alkyl [said lower alkyl has the same meaning as the above-mentioned lower alkyl (i)] or substituted lower alkyl [said lower alkyl has the same meaning as the above-mentioned lower alkyl (i), and the number of the substituent(s), which may be the same or different, is 1 to 3 and examples thereof are halogen, hydroxy, carboxy and

lower alkoxycarbonyl; here, the halogen has the same meaning as the above-mentioned halogen (v) and the lower alkyl moiety of the lower alkoxycarbonyl has the same meaning as the above-mentioned lower alkyl (i)].

The lower alkyl, the aryl, the heterocyclic group moiety of the heterocyclic group and the heterocyclic alkyl, the alkylene moiety of the aralkyl and the heterocylic alkyl, and the aryl moiety of the aralkyl shown here have the same meanings as the above-mentioned lower alkyl (i), aryl (vi), heterocylic group (ix), alkylene moiety (vii) of the aralkyl and aryl moiety (viii) of the aralkyl, respectively. With regard to the substituent in the substituted aryl, the substituted aralkyl, the substituted heterocyclic group and the substituted heterocyclic alkyl, the number of the substituent(s), which may be the same or different, is 1 to 3 and examples thereof are lower alkyl [said lower alkyl has the same meaning as the above-mentioned lower alkyl (i)], lower alkoxy [the lower alkyl moiety of said lower alkoxy has the same meaning as the above-mentioned lower alkyl (i)] and halogen [said halogen has the same meaning as the above-mentioned halogen (v)].

With regard to the quaternary ammonium salt of compound (I), there is no particular limitation so far as it is a quaternary ammonium salt wherein halogenated lower alkyl (said lower alkyl and said halogen have the same meanings as the above-mentioned ones, respectively), halogenated aralkyl (said halogen and said

aralkyl have the same meanings as the above-mentioned ones, respectively), hydroxy lower alkyl (said lower alkyl has the same meaning as the above-mentioned one) or the like is added to a basic moiety of compound (I) and examples thereof are a quaternary ammonium salt prepared by the reaction of compound (I) having a dimethylamino group with methyl iodide, a quaternary ammonium salt prepared by the reaction of compound (I) having a piperidino group with methyl iodide, a quaternary ammonium compound prepared by the reaction of compound (I) having a pyrrolidino group with methyl iodide, a quaternary ammonium salt prepared by compound (I) having a morpholino group with benzyl bromide and a quaternary ammonium salt prepared by exchange of an iodide ion with a hydroxide ion in a quaternary ammonium salt prepared by the reaction of compound (I) having a pyrrolidino group with ethyl iodide.

With regard to the pharmaceutically acceptable salt of compound (I), that which has no toxicity and is soluble in water is preferred and examples thereof are acid addition salts such as inorganic salts including hydrochloride, hydrobromide, nitrate, sulfate, phosphate, etc. and organic salts including benzenesulfonate, benzoate, citrate, fumarate, gluconate, lactate, maleate, malate, oxalate, methanesulfonate, tartrate, etc., alkaline metal salts such as sodium salt and potassium salt etc., alkaline earth metal salt such as magnesium salt and calcium salt etc., metal salts such as aluminum salt and

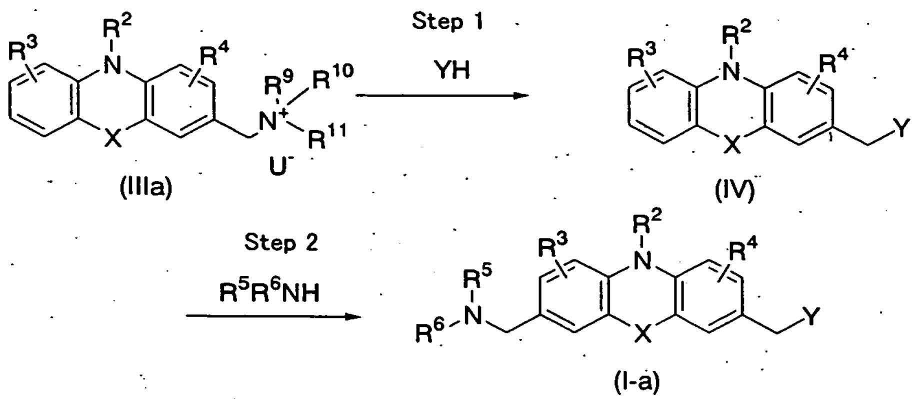
zinc salt, ammonium salt such as ammonium and tetramethylammonium etc., organic amine addition salts such as morpholine addition salt and piperidine addition salt etc. and amino acid addition salt such as glycine addition salt, phenylalanine addition salt, lysine addition salt, aspartic acid addition salt and glutamic acid addition salt etc.

Process for the production of compound (I) will be illustrated as hereunder.

In the process for the production as mentioned below, when the defined group changes under a reaction condition or when it is not appropriate for conducting the process, the production may be easily carried out by subjecting to a method commonly used in synthetic organic chemistry such as by means of protection and deprotection of functional group [e.g., Protective Groups in Organic Synthesis, the third edition, by T. W. Greene and Peter G. M. Wuts, John Wiley & Sons, Inc. (1999)]. If necessary, order of reaction steps such as introduction of substituents may be changed.

Compound (I-a) may be prepared by the production process as shown below.

Production Process 1



(wherein R^2, R^3, R^4, R^5, R^6, X and Y each have the same meaning as defined above, respectively; R^9 represents lower alkyl, allyl or benzyl; R^{10} and R^{11} are the same or different and each represents lower alkyl or cycloalkyl, or R^{10} and R^{11} may form a heterocyclic group together with the adjacent nitrogen atom; and U represents halogen, alkoxysulfonyloxy, aryloxysulfonyloxy, alkylsulfonyloxy or arylsulfonyloxy.)

In the above definitions, the lower alkyl, the cycloalkyl and the halogen each have the same meaning as that mentioned above, respectively. The alkyl moiety of the alkoxysulfonyloxy and the alkylsulfonyloxy and the aryl moiety of the aryloxysulfonyloxy and the arylsulfonyloxy each have the same as the above-mentioned lower alkyl and aryl, respectively. The heterocyclic group formed together with the adjacent nitrogen atom has the same meaning as the above-mentioned one.

<Step 1>

Compound (IIIa) is used as a starting material and is made to react with from one equivalent to a large excess of

YH (wherein Y has the same meaning as defined above) by a process disclosed in the JP-A-7-61983 to give compound (IV). Incidentally, compound (IIIa) is able to be synthesized by the process disclosed in the JP-A-7-61983 or a process similar thereto.

<Step 2>

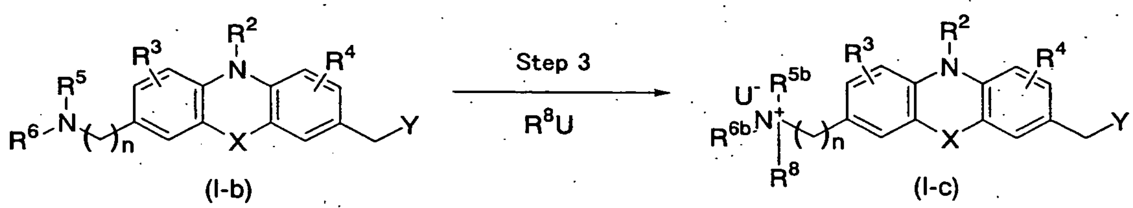
Compound (IV) is made to react with one equivalent to a large excess of R^5R^6NH (wherein R^5 and R^6 each have the same meaning as defined above, respectively) or a hydrochloride thereof in an inert solvent in the presence of one equivalent to a large excess of aqueous formaldehyde solution to give compound (I-a). It is also possible to use a substance equivalent to formaldehyde such as trioxymethylene and paraformaldehyde instead of an aqueous formaldehyde solution.

Since the reaction usually well proceeds under an acidic condition, it is preferred to add an acid such as hydrochloric acid, acetic acid or trifluoroacetic acid to the reaction system if necessary. The reaction is usually carried out at the temperature between 0°C and the boiling point of the solvent used for the reaction, preferably, from room temperature to 80°C and finishes within from 5 minutes to 100 hours. With regard to the inert solvent, water, methanol, ethanol, acetic acid, trifluoroacetic acid, dichloroethane, chloroform, tetrahydrofuran, dimethylacetamide, dimethylformamide, acetone or the like may be used either solely or as the mixture

thereof. Preferably, a mixed solvent of chloroform and acetic acid is used.

Compound (I-c) is produced from compound (I-b) by a process as shown below.

Production Process 2



(wherein R^2, R^3, R^4, R^5, R^6, R^{5b}, R^{6b}, R^8, U, n, X and Y each have the same meaning as defined above, respectively.)

<Step 3>

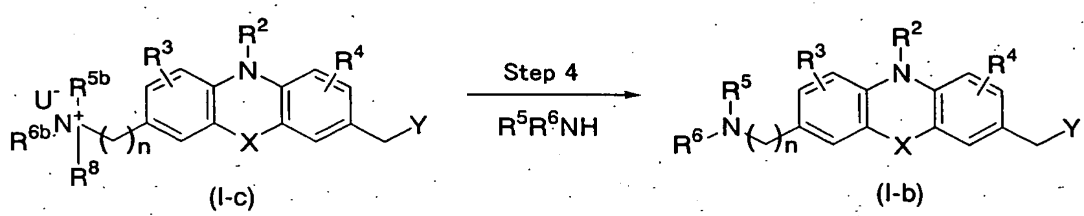
Compound (I-b) is made to react with from one equivalent to a large excess of R^8U (wherein R^8 and U each have the same meaning as defined above, respectively) in an inert solvent for 1 to 48 hour(s) at the temperature of usually from -10°C to the boiling point of the solvent used for the reaction, preferably, at room temperature to give compound (I-c).

With regard to the inert solvent, it is possible to use, for example, water, methanol, ethanol, benzene, toluene, xylene, ethyl acetate, hexane, acetonitrile, dichloromethane, dichloroethane, chloroform, carbon tetrachloride, 1,4-dioxane, tetrahydrofuran, dimethylacetamide, dimethylformamide,

acetone or the like either solely or as the mixture thereof. Preferably, ethyl acetate, dichloroethane, chloroform, etc. are used.

Compound (I-b) is able to be produced from compound (I-c) by the process as shown below.

Producing Process 3



(wherein R^2, R^3, R^4, R^5, R^6, R^{5b}, R^{6b}, U, n, X and Y each have the same meaning as defined above, respectively.)

<Step 4>

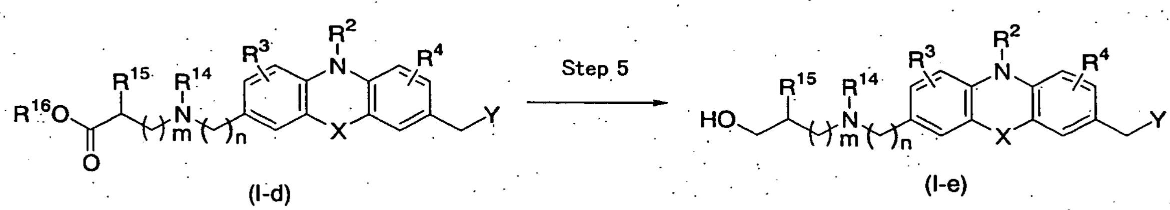
Compound (I-c) is made to react with from one equivalent to a large excess of R^5R^6NH (wherein R^5 and R^6 each have the same meaning as defined above, respectively) in an inert solvent for 1 to 100 hour(s) at the temperature of usually from -10°C to the boiling point of the solvent used for the reaction, preferably, at the temperature between 20°C and 100°C to give compound (I-b).

With regard to the inert solvent, it is possible to use, for example, water, methanol, ethanol, benzene, toluene, xylene, ethyl acetate, hexane, acetonitrile, dichloromethane,

dichloroethane, chloroform, carbon tetrachloride, 1,4-dioxane, tetrahydrofuran, dimethylacetamide, dimethylformamide, acetone or the like either solely or as the mixture thereof. Preferably, chloroform, dimethylformamide, etc. are used. Since the reaction usually well proceeds under a basic condition, it is desirable to add an appropriate base to a reaction system if necessary. With regard to the base, triethylamine, diisopropylethylamine, pyridine, N-methylmorpholine, potassium carbonate, sodium hydride, potassium hydride, calcium hydride, diisopropylethylamine, 1,8-diazabicyclo[5.4.0]undec-7-ene, etc. may be used and, among them, triethylamine is preferred.

Compound (I-e) is able to be produced by the process mentioned below using compound (I-d) in compound (I-b).

Producing Process 4



(wherein R^2, R^3, R^4, n, X and Y each have the same meaning as defined above, respectively; R^{14} and R^{15} are the same or different and each represents hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted lower alkenyl,

substituted or unsubstituted lower alkynyl, substituted or unsubstituted aralkyl or substituted or unsubstituted heterocyclic alkyl, or R^{14} and R^{15} may form a substituted or unsubstituted heterocycle together with the adjacent $CH(CH_2)_mN$; R^{16} represents hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aralkyl or substituted or unsubstituted aryl; and m represents an integer of 0 to 3)

The lower alkyl, the cycloalkyl, the lower alkenyl, the lower alkynyl, the aralkyl, the heterocyclic alkyl and the aryl each have the same meaning as defined above, respectively and the substituents thereof also have the same meanings as defined above, respectively.

With regard to the substituted or unsubstituted heterocycle formed from R^{14} and R^{15} together with the adjacent $CH(CH_2)_mN$ thereto, tetrahydropyridine, pyrrolidine, piperidine, homopiperidine, piperazine, homopiperazine, morpholine, thiomorpholine, perhydroazepine, perhydroazocine, tetrahydroquinoline and tetrahydroisoquinoline, or the like are exemplified and the substituent thereof has the same meaning as the substituent for the above-mentioned heterocyclic group formed together with the adjacent nitrogen atom.

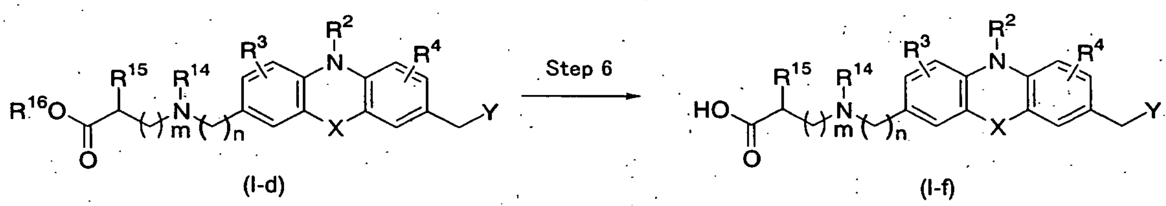
<Step 5>

When compound (I-d) is treated for 10 minutes to 24 hours, preferably, 1 to 3 hour(s) in the presence of 2 to 4 equivalents of a reducing agent such as lithium aluminum hydride, diisopropyl lithium aluminum hydride, etc., preferably, diisopropyl lithium aluminum hydride usually at the temperature from -78°C to 40°C in an inert solvent, compound (I-e) is able to be prepared.

With regard to the inert solvent, dichloromethane, chloroform, carbon tetrachloride, dichloroethane, benzene, toluene, xylene, tetrahydrofuran, diethyl ether, etc. are used either solely or as the mixture thereof and, preferably, dichloromethane or toluene is used.

Compound (I-f) is able to be produced from compound (I-d) by the process as mentioned below.

Producing Process 5



(wherein R^2, R^3, R^4, R^{14}, R^{15}, R^{16}, n, X, Y and m each have the same meaning as defined above, respectively.)

<Step 6>

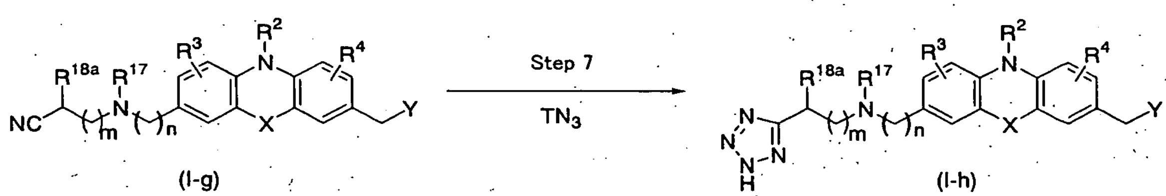
When compound (I-d) is treated for 1 to 48 hours, preferably, 1 to 3 hour(s) in the presence of from one equivalent to a large excess of appropriate base at the temperature of usually from

0°C to the boiling point of the solvent used for the reaction, preferably, at the temperature between room temperature and 100°C in an inert solvent, compound (I-f) is able to be produced.

With regard to the appropriate base, sodium hydroxide, lithium hydroxide, potassium hydroxide, potassium carbonate, cesium carbonate and sodium methoxide are exemplified and sodium hydroxide is preferably exemplified. With regard to the inert solvent, water, tetrahydrofuran, diethyl ether, methanol, ethanol, propanol, dichloromethane, dichloroethane, benzene, toluene, xylene, etc. may be used either solely or as the mixture thereof and, preferably, tetrahydrofuran or methanol or a mixed solvent thereof with water is used.

Compound (I-h) is able to be produced by the following process from compound (I-g) in compound (I-c).

Producing Process 6



(wherein R^2, R^3, R^4, n, X, Y and m each have the same meaning as defined above, respectively; R^{17} and R^{18a} each have the same meaning as the above R^{14} and the above R^{15}, respectively; and T represents alkaline metal, ammonium, trialkylsilyl or trialkyltin.)

The alkyl in the trialkylsilyl and trialkyltin in the above definition has the same meaning as the above-mentioned lower alkyl. Examples of the alkaline metal are sodium, potassium or the like.

<Step 7>

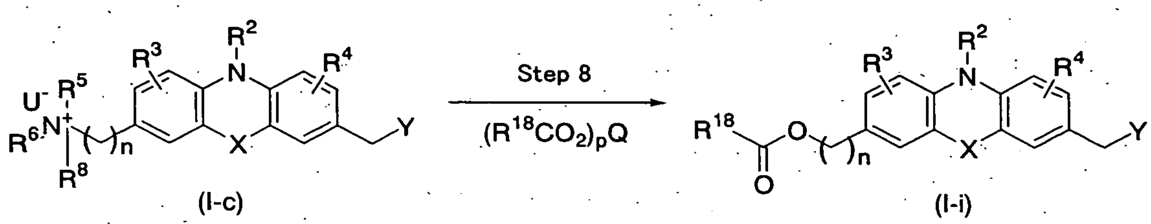
When compound (I-g) is made to react with from one equivalent to a large excess, preferably, 2 to 4 equivalents of TN_3 (wherein T has the same meaning as defined above) for 1 to 200 hours, preferably, for 3 to 48 hours usually in the presence of from catalytic amount to a large excess, preferably, 0.5 to 2 equivalent(s) of appropriate additive for accelerating the reaction at the temperature of from 0°C to the boiling point of the solvent used for the reaction, preferably, at the temperature between room temperature and 200°C in an inert solvent, compound (I-h) is able to be produced.

Examples of the appropriate additive are silicon tetrachloride, lithium chloride, aluminum chloride, ammonium chloride, trialkyltin chloride, dialkyltin oxide, trialkyl aluminum, triethylamine hydrochloride, triethylamine hydrobromide, sodium hydroxide, potassium tert-butoxide, sodium hydroxide, and zinc bromide and preferred examples are ammonium chloride and dialkyltin oxide. With regard to the inert solvent, water, acetonitrile, dimethylformamide, dimethylacetamide, N-methyl-2-pyrrolidone, dimethyl sulfoxide, acetic acid, glacial acetic acid, tetrahydrofuran,

benzene, toluene, xylene, etc. may be used either solely or as the mixture thereof. Preferably, dimethylformamide or toluene is used.

Compound (I-i) is able to be produced by the following process from compound (I-c).

Producing Process 7



(wherein R^2, R^3, R^4, R^5, R^6, R^8, U, n, X and Y each have the same meaning as defined above, respectively; R^{18} represents substituted or unsubstituted lower alkyl; Q represents alkaline metal or alkaline earth metal and, when Q represents alkaline metal, p represents 1, while, when Q represents alkaline earth metal, p represents 2)

In the above definition, the alkaline metal has the same meaning as the above-mentioned alkaline metal and examples of the alkaline earth metal are magnesium, calcium or the like.

<Step 8>

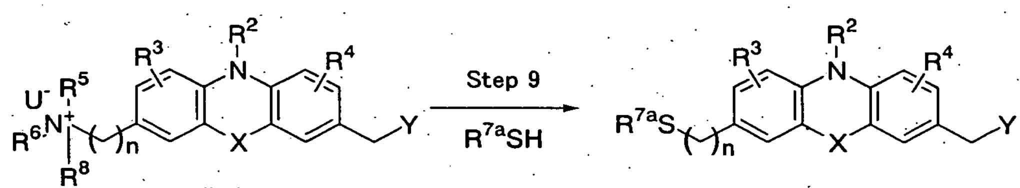
When compound (I-c) is made to react with from 1 equivalent to a large excess, preferably, 4 to 8 equivalents of $(R^{18}CO_2)_pQ$ (wherein R^{18}, Q and p each have the same meaning as defined above,

respectively) for 1 to 100 hour(s), preferably, 3 to 72 hours at the temperature between 0°C and the boiling point of the solvent used for the reaction, preferably, the temperature between 70°C and 80°C in an inert solvent, compound (I-i) is able to be produced.

With regard to the inert solvent, dimethylacetamide, N-methyl-2-pyrrolidone, dimethyl sulfoxide, etc. may be used either solely or as the mixture thereof and, preferably, dimethyl sulfoxide is used.

Compound (I-j) is able to be produced from compound (I-c) by the process as shown below.

Production Process 8



(wherein R^2, R^3, R^4, R^5, R^6, R^{7a}, R^8, U, n, X and Y each have the same meaning as defined above, respectively.)

<Step 9>

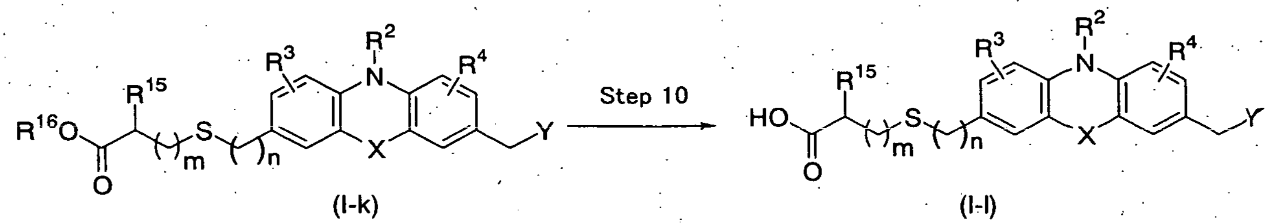
When compound (I-c) is made to react with from 1 equivalent to a large excess, preferably, 2 to 8 equivalents of $R^{7a}SH$ (wherein R^{7a} has the same meaning as defined above) for 1 to 100 hour(s), preferably, 3 to 72 hours in the presence of 1 equivalent to

a great excess, preferably, 1 to 3 equivalent(s) of an appropriate base at the temperature between 0°C to the boiling point of a solvent used for the reaction, preferably, between 30°C and 80°C in an inert solvent, compound (I-j) is able to be produced.

With regard to the appropriate base, triethylamine, diisopropylethylamine, pyridine, N-methylmorpholine, potassium carbonate, sodium hydride, potassium hydride, calcium hydride, diisopropylethylamine, 1,8-diazabicyclo[5.4.0]undec-7-ene, etc. may be used and, among them, 1,8-diazabicyclo[5.4.0]undec-7-ene is preferred. With regard to the inert solvent, dichloromethane, chloroform, carbon tetrachloride, dichloroethane, benzene, toluene, xylene, ethyl acetate, dimethylacetamide, N-methyl-2-pyrrolidone, dimethyl sulfoxide, etc. may be used either solely or as the mixture thereof and, preferably, chloroform may be used.

Compound (I-l) is able to be produced by the process as mentioned below from compound (I-k) in compound (I-j).

Production Process 9



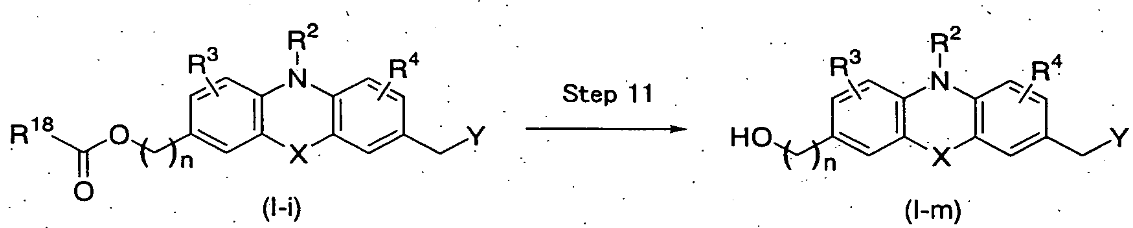
(wherein R^2, R^3, R^4, R^{15}, R^{16}, m, n, X and Y each have the same meaning as defined above, respectively.)

<Step 10>

Compound (I-l) is able to be produced from compound (I-k) by conducting the reaction similar to the step 6 for Production Process 5.

Compound (I-m) is able to be produced from compound (I-i) by the process as mentioned below.

Production Process 10



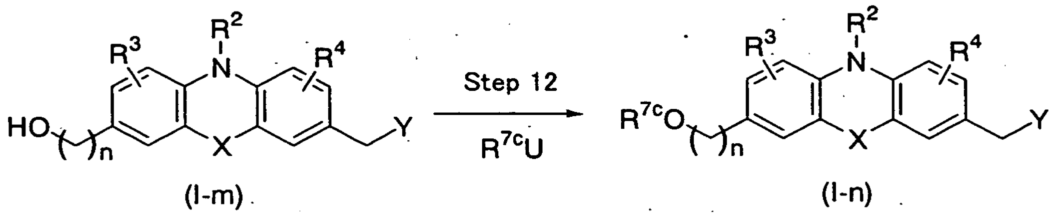
(wherein R^2, R^3, R^4, R^{18}, n, X and Y each have the same meaning as defined above, respectively.)

<Step 11>

Compound (I-m) is able to be produced from compound (I-i) by conducting the reaction similar to the step 6 for Production Process 5.

Compound (I-n) is able to be produced from compound (I-m) by the process as mentioned below.

Production Process 11



(wherein R^2, R^3, R^4, U, n, X and Y each have the same meaning as defined above, respectively, and R^{7c} represents a group wherein hydrogen is removed from the definition of the above R^7.)

<Step 12>

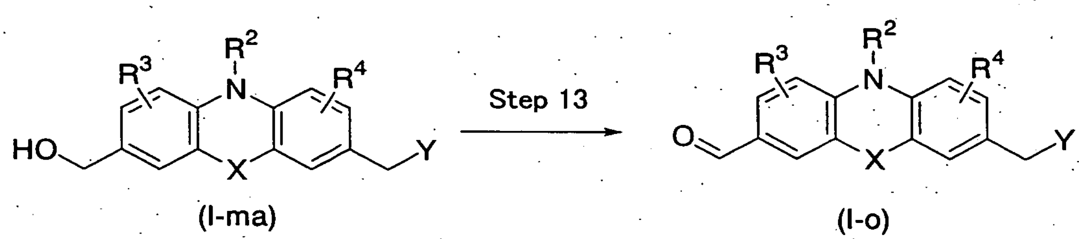
When compound (I-m) is made to react with from one equivalent to a large excess, preferably, from 1 to 3 equivalent(s) of $R^{7c}U$ (wherein R^{7c} and U each have the same meaning as defined above, respectively) for 1 to 48 hour(s), preferably, 3 to 24 hour(s) in the presence of from 1 equivalent to a large excess, preferably, 1 to 3 equivalent(s) of an appropriate base, at the temperature between 0°C and the boiling point of the solvent used for the reaction, preferably, at the temperature between room temperature and 80°C in an insert solvent, compound (I-n) is able to be prepared.

Examples of the appropriate base are potassium carbonate, sodium hydride, potassium hydride, calcium hydride and lower alkyllithium and preferred ones are sodium hydride, potassium hydride, etc. With regard to the inert solvent, dichloromethane, chloroform, carbon tetrachloride, dichloroethane, benzene,

toluene, xylene, ethyl acetate, dimethylformamide, dimethylacetamide, N-methyl-2-pyrrolidone, tetrahydrofuran, diethyl ether, etc. may be used either either solely or as the mixture thereof. Preferably, chloroform is used.

Compound (I-o) is able to be produced by the process as mentioned below from compound (I-ma) which is compound (I-m) wherein n is 1.

Producing Process 12



(wherein R^2, R^3, R^4, X and Y each have the same meaning as defined above, respectively.)

<Step 13>

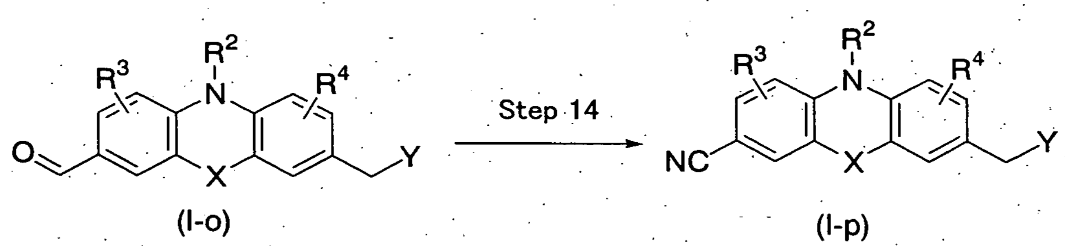
When compound (I-ma) is treated for 1 to 48 hour(s), preferably, 3 to 24 hours in the presence of from 1 equivalent to a large excess, preferably, 3 to 6 equivalents of an appropriate oxidizing agent at the temperature between 0°C and the boiling point of the solvent used for the reaction, preferably, the temperature between room temperature and 60°C in an inert solvent, compound (I-o) is able to be prepared.

With regard to the appropriate oxidizing agent, manganese

dioxide, chromic acid, pyridinium chlorochromate, pyridinium dichromate, potassium permanganate, sulfur trioxide-pyridine and oxone are exemplified and manganese dioxide is preferably exemplified. With regard to the inert solvent, dichloromethane, chloroform, carbon tetrachloride, dichloroethane, benzene, toluene, xylene, ethyl acetate, acetic acid, propionic acid, butyric acid, trifluoroacetic acid, water, pyridine, dimethylformamide, dimethylacetamide, N-methyl-2-pyrrolidone, 1,4-dioxane, tetrahydrofuran, diethyl ether, etc. may be used either solely or as the mixture thereof. Preferably, dimethylformamide, tetrahydrofuran, etc. may be used.

Compound (I-p) is able to be produced by the process as mentioned below from compound (I-o).

Producing Process 13



(wherein R^2, R^3, R^4, X and Y each have the same meaning as defined above, respectively.)

<Step 14>

When compound (I-o) is made to react with from 1 equivalent

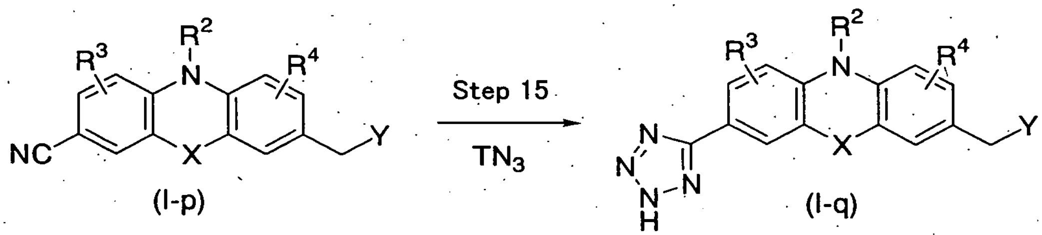
to a large excess, preferably, 1 to 3 equivalent(s) of hydroxylamine or its hydrochloride, sulfate, p-toluenesulfonate, etc. thereof, O-phenylcarbamylhydroxylamine or its hydrochloride, sulfate, p-toluenesulfonate, etc. thereof or N-hydroxybenzamide, preferably, with hydroxylamine for 1 to 48 hour(s), preferably, 3 to 24 hours at the temperature between 0°C and the boiling point of the solvent used for the reaction, preferably, between room temperature and 90°C in an inert solvent, compound (I-p) is able to be prepared. If necessary, addition of 1 equivalent to a large excess, preferably, 1 to 3 equivalent(s) of an appropriate dehydrating agent, addition of 1 equivalent to a large excess, preferably, 2 to 6 equivalents of an appropriate base or irradiation with microwave may be carried out.

With regard to the appropriate dehydrating agent, acetic anhydride, phthalic anhydride, sodium hydrogen sulfate, oxone, sodium formate, dialkyltin oxide, alumina, silica gel, sodium acetate, formamide, diphosphorus pentaoxide, ferric chloride, formic acid, acetic acid, propionic acid, phosphorus oxychloride, p-toluenesulfonic acid, etc. may be exemplified and, preferably, acetic anhydride, phthalic anhydride, etc. may be exemplified. With regard to the appropriate base, triethylamine, pyridine, sodium hydride, potassium hydride, etc. may be exemplified and, preferably, triethylamine or pyridine may be exemplified.

With regard to the inert solvent, dichloromethane, chloroform, carbon tetrachloride, dichloroethane, benzene, toluene, xylene, nitrobenzene, acetonitrile, ethyl acetate, acetic acid, propionic acid, butyric acid, trifluoroacetic acid, pyridine, dimethylformamide, dimethylacetamide, N-methyl-2-pyrrolidone, 1,4-dioxane, tetrahydrofuran, diethyl ether, methanol, ethanol, propanol, etc. may be used either solely or as the mixture thereof. Preferably, acetonitrile, dimethylformamide, etc. may be used.

Compound (I-q) is able to be produced by the process as mentioned below from compound (I-p).

Producing Process 14



(wherein R^2, R^3, R^4, T, X and Y each have the same meaning as define above, respectively.)

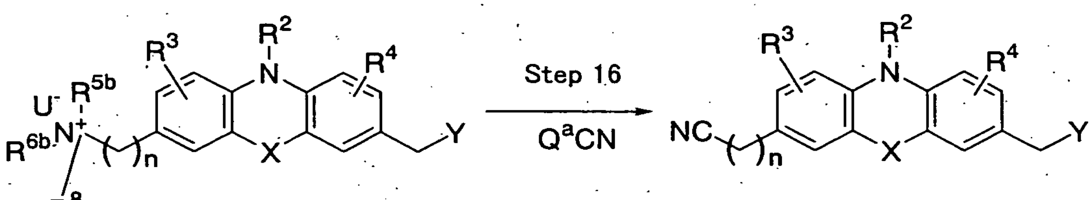
<Step 15>

Compound (I-q) is able to be produced using compound (I-p) by conducting the reaction similar to that in the step 7 of Producing Process 6.

Compound (I-r) is able to be produced by the process as

mentioned below from compound (I-c)

Producing Process 15



(I-c) (I-r)

(wherein R^2, R^3, R^4, R^{5b}, R^{6b}, R^8, U, n, X and Y each have the same meaning as defined above, respectively; and Q^a represents the same alkaline metal as defined above.)

<Step 16>

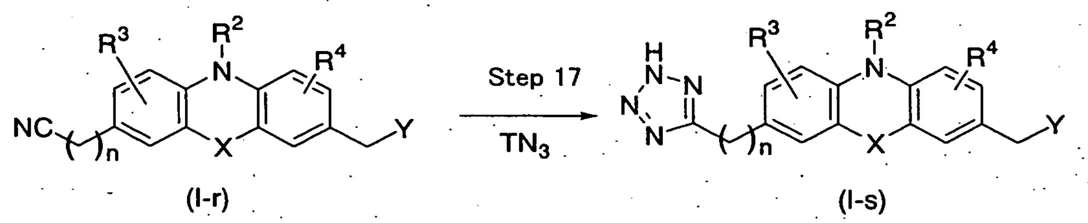
When compound (I-c) is made to react with from 1 equivalent to a large excess, preferably, 2 to 4 equivalents of Q^aCN (wherein Q^a has the same meaning as defined above), preferably sodium cyanide for 1 to 48 hour(s), preferably, 3 to 24 hours at the temperature between room temperature and the boiling point of the solvent used for the reaction, preferably, between 40°C and 80°C in an inert solvent, compound (I-r) is able to be produced.

With regard to the inert solvent, dichloromethane, chloroform, carbon tetrachloride, dichloroethane, benzene, toluene, xylene, dimethylformamide, dimethylacetamide, N-methyl-2-pyrrolidone, 1,4-dioxane, tetrahydrofuran, etc. may be used either solely or as the mixture thereof. Preferably,

dimethylformamide, etc. may be used.

Compound (I-s) is able to be produced by the process as shown below from compound (I-r).

Producing Process 16



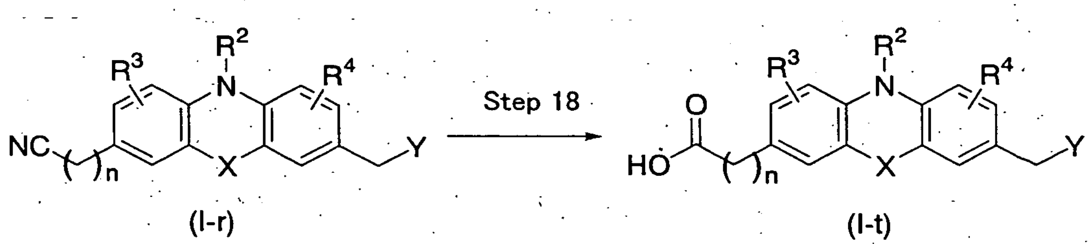
(wherein R^2, R^3, R^4, T, n, X and Y each have the same meaning as defined above, respectively.)

<Step 17>

Compound (I-s) is able to be produced by conducting the reaction similar to the step 7 of Producing Process 6 using compound (I-r).

Compound (I-t) is able to be produced by the process as mentioned below from compound (I-r).

Producing Process 17



(wherein R^2, R^3, R^4, n, X and Y each have the same meaning

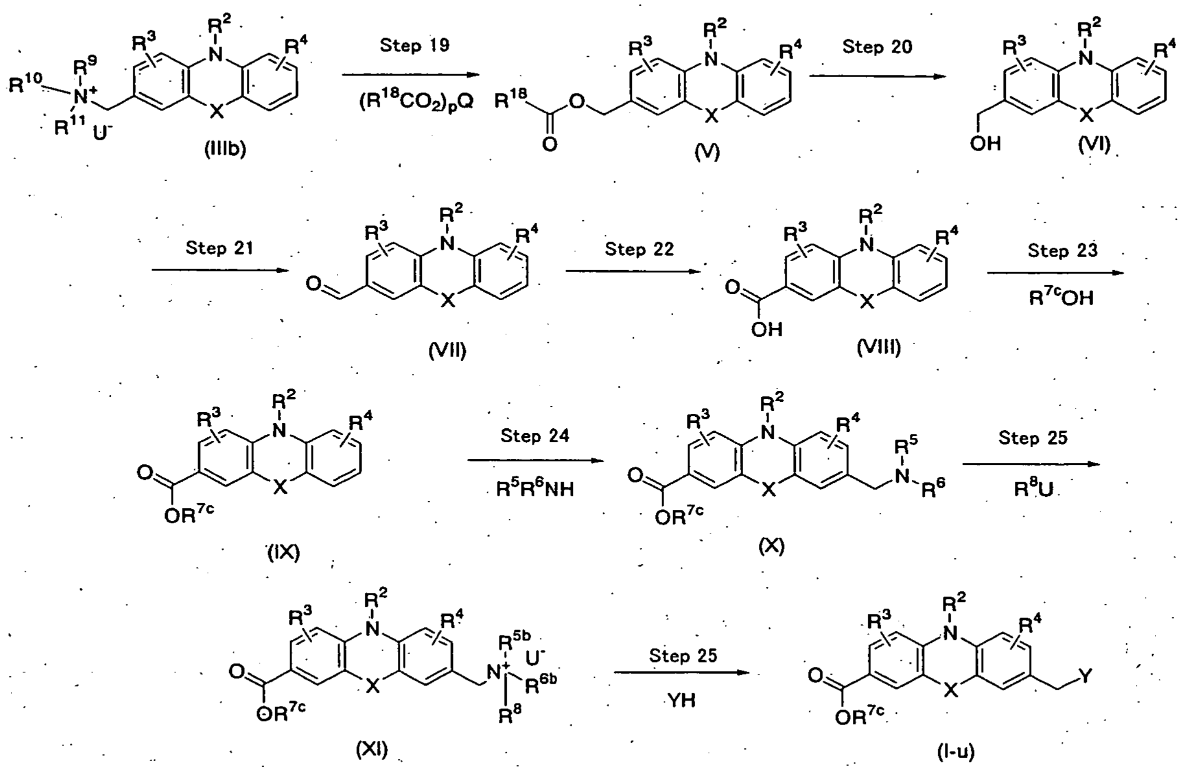
as defined above, respectively.)

<Step 18>

Compound (I-t) is able to be produced by conducting the reaction similar to the step 6 of Producing Process 5 using compound (I-r).

Compound (I-u) is able to be produced by the process as mentioned below from compound (IIIb).

Producing Process 18



(wherein R^2, R^3, R^4, R^5, R^6, R^{5b}, R^{6b}, R^{7c}, R^8, R^9, R^{10}, R^{11}、R^{18}, Q, p, U, X and Y each have the same meaning as defined above, respectively.)

<Step 19>

Compound (V) is able to be produced by conducting the reaction similar to the step 8 of Producing Process 7 using compound (IIIb).

<Step 20>

Compound (VI) is able to be produced by conducting the reaction similar to the step 6 of Producing Process 5 using compound (V).

<Step 21>

Compound (VII) is able to be produced by conducting the reaction similar to the step 13 of Producing Process 12 using compound (VI).

<Step 22>

When compound (VII) is treated for 10 minutes to 24 hours, preferably, 1 to 4 hour(s) in the presence of 2 to 4 equivalent(s) of an oxidizing agent such as silver nitrate, silver (I) oxide, silver (II) oxide, chromic acid, pyridinium chlorochromate, pyridinium dichlorochromate, potassium permanganate, sodium periodate, sodium perchlorate, hydrogen peroxide and sodium chlorite, preferably, silver nitrate or sodium perchlorate usually at the temperature between 0°C and 80°C in an inert solvent, compound (VIII) is able to be produced. If necessary, 0.1 to 4 equivalent(s) of an organic substance such as acetic acid or an inorganic substance such as sulfuric acid, sodium dihydrogen phosphate, sulfamic acid and ruthenium oxide may

be added as an additive.

With regard to the inert solvent, diethyl ether, tetrahydrofuran, 1,4-dioxane, dimethylformamide, dimethylacetamide, dimethyl sulfoxide, benzene, toluene, xylene, dichloromethane, chloroform, 1,2-dichloroethane, acetonitrile, ethyl acetate, methyl acetate, methyl ethyl ketone, hydrochloric acid, acetic acid, acetic anhydride, sulfuric acid, water, etc. may be exemplified and, preferably, acetonitrile, water, etc. are exemplified. Each of them may be used solely or as the mixture thereof.

<Step 23>

When compound (VIII) is made to react with 1 to 20 equivalent(s) of halogenating agent for 10 minutes to 24 hours usually at the temperature between 0°C and 80°C, preferably, at room temperature in an inert solvent and, after that, it is made to react with from 1 equivalent to a large excess of $R^{7c}OH$ (wherein R^{7c} has the same meaning as defined above), compound (IX) is able to be produced.

With regard to the halogenating agent, thionyl chloride, oxalyl chloride and phosphorus oxychloride are exemplified and, preferably, thionyl chloride is exemplified. With regard to the inert solvent, dichloromethane, chloroform, tetrahydrofuran, dimethylformamide, dimethylacetamide, 1,4-dioxane, acetonitrile, benzene, toluene and xylene are exemplified and each of them may be used either solely or as

the mixture thereof. With regard to the inert solvent, dichloromethane is preferably exemplified.

<Step 24>

Compound (X) is able to be produced by conducting the reaction similar to the step 2 of Producing Process 1 using compound (IX).

<Step 25>

Compound (XI) is able to be produced by conducting the reaction similar to the step 3 of Producing Process 2 using compound (X).

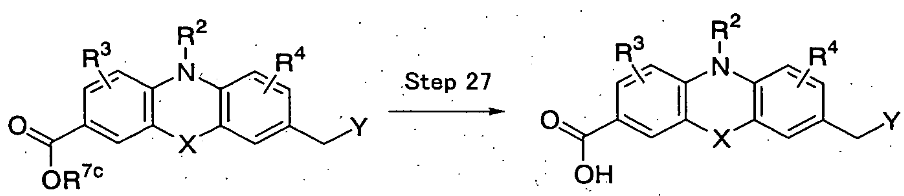
<Step 26>

Compound (I-u) is able to be produced by conducting the reaction similar to the step 1 of Producing Process 1 using compound (XI).

Compound (I-v) is able to be produced by the process as shown below from compound (I-u).

Producing Process 19

(step 27)



(I-u) (I-v)

(wherein R^2, R^3, R^4, R^{7c}, X and Y each have the same meaning

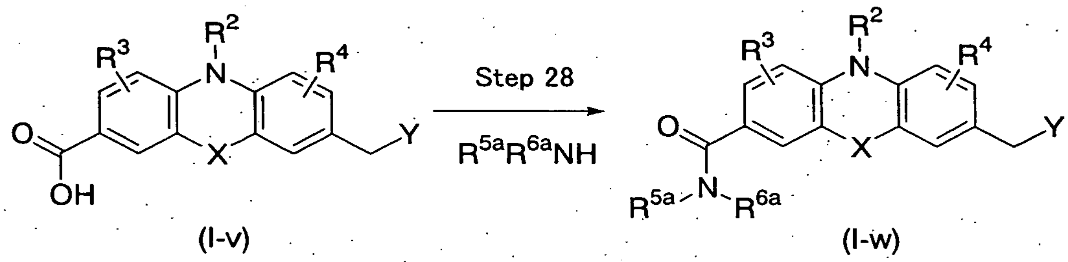
as defined above, respectively.)

<Step 27>

Compound (I-v) is able to be produced by conducting the reaction similar to the step 6 of Producing Process 5 using compound (I-u).

Compound (I-w) is able to be produced by the process as shown below from compound (I-v).

Producing Process 20



(wherein R^2, R^3, R^4, R^{5a}, R^{6a}, X and Y each have the same meaning as defined above, respectively.)

<Step 28>

When compound (I-v) is made to react with 1 to 20 equivalent(s) of a halogenating agent for 10 minutes to 24 hours usually at the temperature between 0°C and 80°C, preferably, at room temperature in an inert solvent and, after that, made to react with 1 equivalent to a large excess of $R^{5a}R^{6a}NH$ (wherein R^{5a} and R^{6a} each have the same meaning as defined above, respectively), compound (I-w) is able to be produced. If necessary, 1 equivalent to a large excess of an appropriate

base may be added thereto.

With regard to the halogenating agent, thionyl chloride, oxalyl chloride, phosphorus oxychloride, etc. may be exemplified and, preferably, thionyl chloride is exemplified. With regard to the appropriate base, pyridine, triethylamine, diisopropylethylamine, N-methylmorpholine, etc. may be exemplified and, preferably, triethylamine may be exemplified. With regard to the inert solvent, dichloromethane, chloroform, tetrahydrofuran, dimethylformamide, dimethylacetamide, 1,4-dioxane, acetonitrile, benzene, toluene, xylene, etc. may be exemplified and each of them may be used solely or as the mixture thereof. With regard to the inert solvent, dichloromethane is preferably exemplified.

In the production of compound (I-w), it is also possible to apply the method which has been commonly used in peptide chemistry. Thus, when compound (I-v) is made to react with 1 to 10 equivalent(s) of $R^{5a}R^{6a}NH$ (wherein R^{5a} and R^{6a} each have the same meaning as defined above, respectively) together with 0.5 to 10 equivalent(s) of an appropriate condensing agent usually at the temperature between 0°C and 50°C for 10 minutes to 70 hours in an inert solvent, compound (I-w) is able to be produced.

With regard to the inert solvent, diethyl ether, tetrahydrofuran, 1,4-dioxane, dimethylformamide, dimethylacetamide, dimethyl sulfoxide, benzene, toluene,

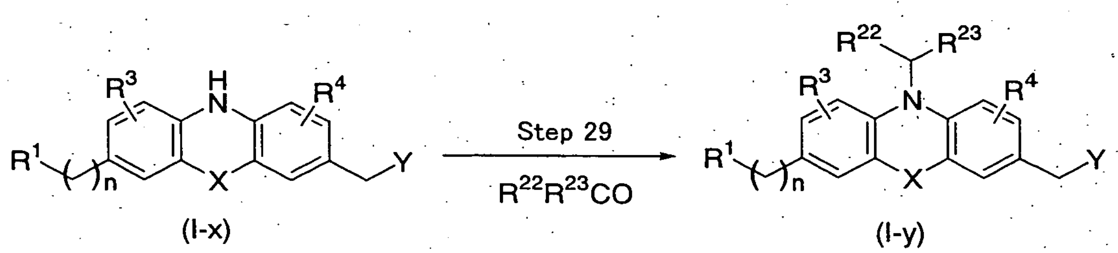
xylene, acetonitrile, ethyl acetate, pyridine, dichloromethane, chloroform and carbon tetrachloride may be exemplified and, preferably, tetrahydrofuran and dimethylformamide may be exemplified.

With regard to the appropriate condensing agent, 1,3-dicyclohexylocarbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide-bonded polystyrene resin (EDC resin), etc. may be exemplified. It is also possible to add an additive such as N-hydroxysuccinimide, 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine, 1-hydroxybenzotriazole, preferably, 1-hydroxybenzotriazole thereto.

EDC resin is able to be produced by a process mentioned in *Tetrahedron Letters*, volume 34, no. 48, page 7685 (1993).

Compound (I-y) is able to be produced by the process shown below from compound (I-x) in compound (I).

Producing Process 21



(wherein R^1, R^3, R^4, n, X and Y each have the same meaning

as defined above, respectively; and R^{22} and R^{23} are the same or different and each represents hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aralkyl or substituted or unsubstituted heterocyclic alkyl.)

In the above definitions, the lower alkyl, the cycloalkyl, the lower alkenyl, the lower alkynyl, the aralkyl and the heterocyclic alkyl each have the same meaning as defined above, respectively and the substituent thereof also has the same meaning as defined above, respectively.

<Step 29>

When compound (I-x) is made to react with 1 equivalent to a large excess, preferably, 1 to 10 equivalent(s) of $R^{22}R^{23}CO$ (wherein R^{22} and R^{23} each have the same meaning as defined above, respectively) usually at the temperature between -78°C and 100°C, preferably, the temperature between 0°C and 50°C in the presence of 1 equivalent to large excess, preferably, 1 to 3 equivalent(s) of an appropriate reducing agent, for 10 minutes to 48 hours in an inert solvent, compound (I-y) is able to be produced.

With regard to the appropriate reducing agent, sodium borohydride, sodium triacetoxyborohydride, sodium cyanoborohydride, etc. may be exemplified and, preferably, sodium cyanoborohydride may be exemplified. If necessary,

catalytic amount to solvent amount, preferably, 0.5 equivalent to solvent amount of appropriate acid may be added thereto. With regard to the appropriate acid, formic acid, acetic acid, trifluoroacetic acid, propionic acid, hydrochloric acid, etc. may be exemplified and, preferably, acetic acid may be exemplified.

With regard to the inert solvent, dichloromethane, chloroform, carbon tetrachloride, dichloroethane, benzene, toluene, xylene, diethyl ether, 1,4-dioxane, dimethylformamide, dimethylacetamide, acetonitrile, hexane, formic acid, acetic acid, trifluoroacetic acid, propionic acid, hydrochloric acid, etc. may be exemplified and each of them may be used solely or as the mixture thereof. Preferably, tetrahydrofuran, acetic acid, etc. may be exemplified.

Transformation of each functional group in compound (I) and the starting compounds and transformation of a functional group contained in the substituent may also be carried out a known method [such as a method mentioned in Comprehensive Organic Transformation, second edition, by R. C. Larock, John Wiley & Sons, Inc. (1999)], etc.

When the above-mentioned method, etc. are carried out in an appropriately combined manner, it is possible to prepare compound (I) having a desired functional group at a desired position.

Isolation and purification of an intermediate and the

product in the above producing process may be carried out by an appropriate combination of the methods which are used in common organic synthesis such as filtration, extraction, washing, drying, concentration, crystallization, various kinds of chromatographies, etc. It is also possible to conduct a purifying method commonly used in general parallel synthetic methods such as purifying methods using scavenger resin and ion-exchange resin. It is also possible that the intermediate is not particularly purified but just subjected to the next reaction.

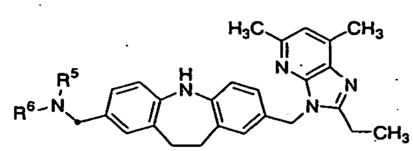
In some of compound (I), there may be isomers such as regioisomer, geometric isomer, optical isomer or tautomer and all possible isomers including the above and mixtures of any ratio of said isomers can be used as an agent for the prevention and/or treatment of asthma.

When a salt of compound (I) is to be prepared, the product may be just purified in case a salt of compound (I) is obtained while, in case compound (I) is obtained in a free form, compound (I) may be dissolved or suspended in an appropriate solvent and isolated and purified after addition of acid or base.

Some of compound (I) or the pharmaceutically acceptable salt thereof may be present in a form of an adduct with water or with various solvents, and such adducts are also able to be used as an agent for prevention and/or treatment of asthma of the present invention.

Although specific examples of compound (I) which is able to be used as an agent for prevention and/or treatment of asthma of the present invention are shown in the following Tables 1 to 13, the scope of the compound used as an agent for prevention and/or treatment of asthma of the present invention is not limited to thereto.

Table 1



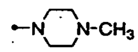
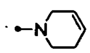
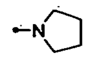
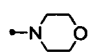
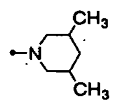
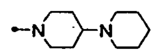
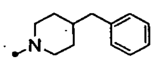
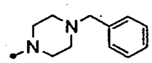
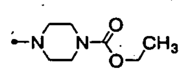
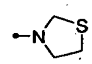
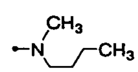
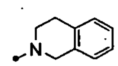
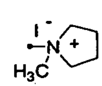
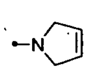
Compound No.	$\bullet$—NR^5R^6
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	

Table 1 (continued)

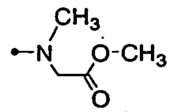
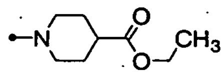
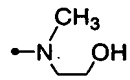
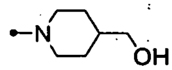
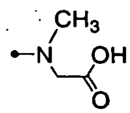
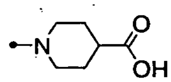
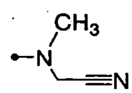
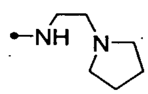
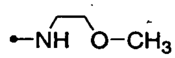
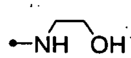
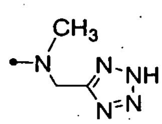
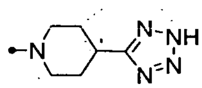
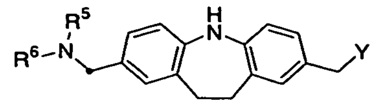
Compound No.	$\bullet\!-\!NR^5R^6$
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	$\bullet\!-\!NH_2$
26	
27	

Table 2



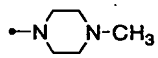
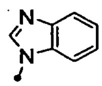
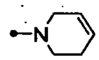
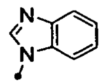
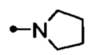
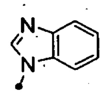
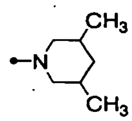
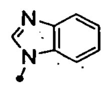
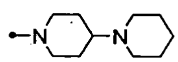
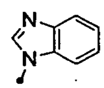
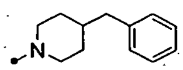
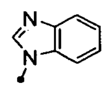
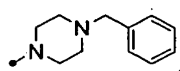
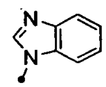
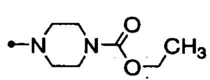
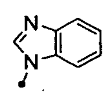
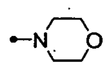
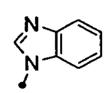
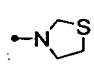
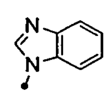
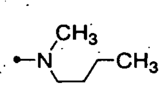
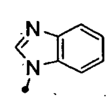
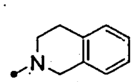
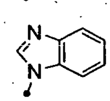
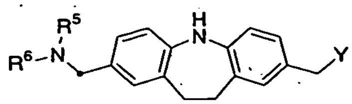
Compound No.	NR⁵R⁶	Y	Mass Spectrometric Data
28			MS m/z 438 $(M+H)^+$
29			MS m/z 421 $(M+H)^+$
30			MS m/z 409 $(M+H)^+$
31			MS m/z 451 $(M+H)^+$
32			MS m/z 506 $(M+H)^+$
33			MS m/z 513 $(M+H)^+$
34			MS m/z 514 $(M+H)^+$
35			MS m/z 496 $(M+H)^+$
36			MS m/z 425 $(M+H)^+$
37			MS m/z 427 $(M+H)^+$
38			MS m/z 425 $(M+H)^+$
39			MS m/z 471 $(M+H)^+$

Table 3



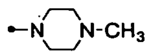
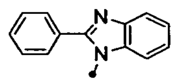
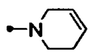
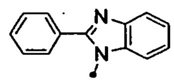
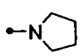
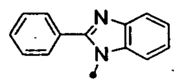
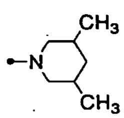
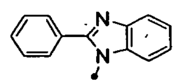
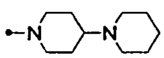
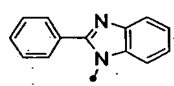
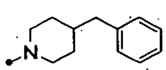
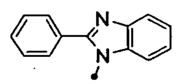
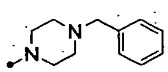
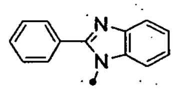
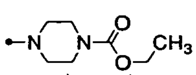
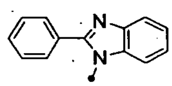
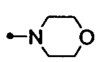
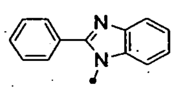
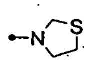
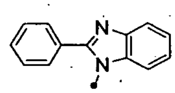
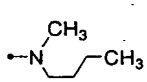
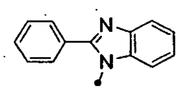
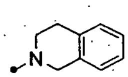
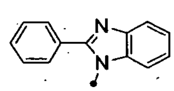
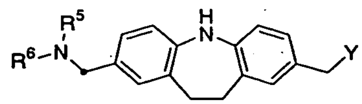
Compound No.	•—NR^5R^6	•—Y	Mass Spectrometric Data
40			MS m/z 514 $(M+H)^+$
41			MS m/z 497 $(M+H)^+$
42			MS m/z 485 $(M+H)^+$
43			MS m/z 527 $(M+H)^+$
44			MS m/z 582 $(M+H)^+$
45			MS m/z 589 $(M+H)^+$
46			MS m/z 590 $(M+H)^+$
47			MS m/z 572 $(M+H)^+$
48			MS m/z 501 $(M+H)^+$
49			MS m/z 503 $(M+H)^+$
50			MS m/z 501 $(M+H)^+$
51			MS m/z 547 $(M+H)^+$

Table 4



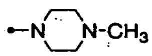
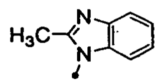
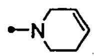
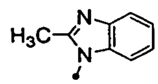
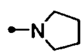
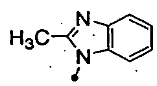
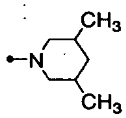
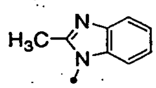
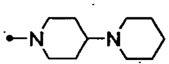
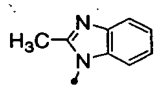
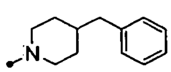
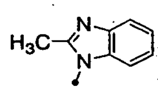
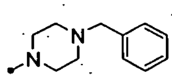
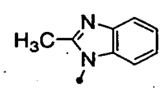
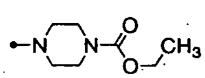
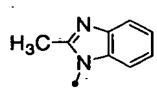
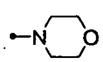
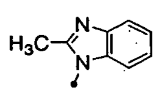
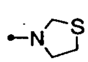
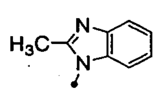
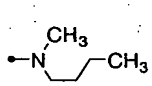
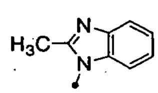
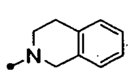
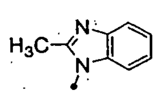
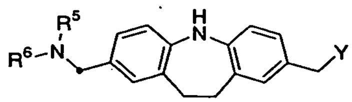
Compound No.	•—NR^5R^6	•—Y	Mass Spectrometric Data
52			MS m/z 452 $(M+H)^+$
53			MS m/z 435 $(M+H)^+$
54			MS m/z 423 $(M+H)^+$
55			MS m/z 465 $(M+H)^+$
56			MS m/z 520 $(M+H)^+$
57			MS m/z 527 $(M+H)^+$
58			MS m/z 528 $(M+H)^+$
59			MS m/z 510 $(M+H)^+$
60			MS m/z 439 $(M+H)^+$
61			MS m/z 441 $(M+H)^+$
62			MS m/z 439 $(M+H)^+$
63			MS m/z 485 $(M+H)^+$

Table 5



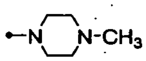
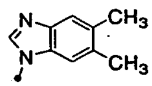
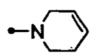
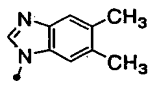
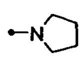
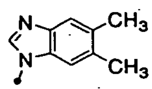
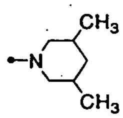
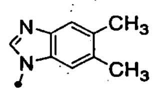
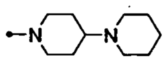
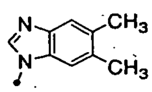
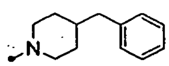
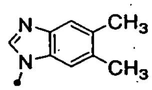
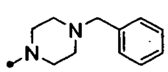
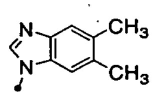
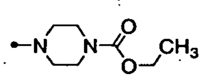
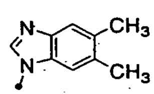
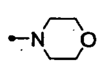
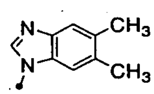
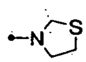
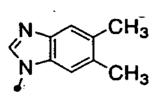
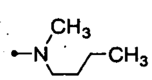
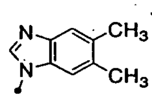
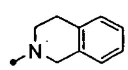
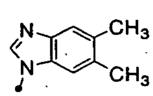
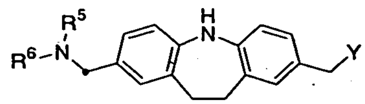
Compound No.	•—NR^5R^6	•—Y	Mass Spectrometric Data
64			MS m/z 466 $(M+H)^+$
65			MS m/z 449 $(M+H)^+$
66			MS m/z 437 $(M+H)^+$
67			MS m/z 479 $(M+H)^+$
68			MS m/z 534 $(M+H)^+$
69			MS m/z 541 $(M+H)^+$
70			MS m/z 542 $(M+H)^+$
71			MS m/z 524 $(M+H)^+$
72			MS m/z 453 $(M+H)^+$
73			MS m/z 455 $(M+H)^+$
74			MS m/z 453 $(M+H)^+$
75			MS m/z 499 $(M+H)^+$

Table 6



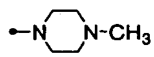
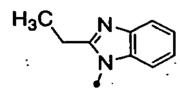
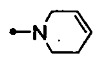
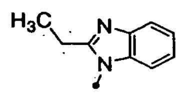
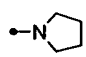
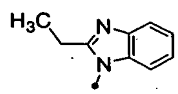
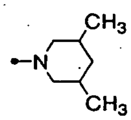
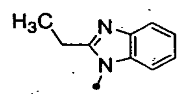
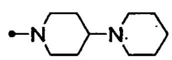
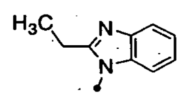
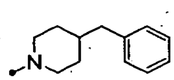
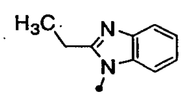
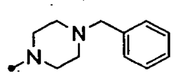
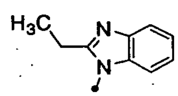
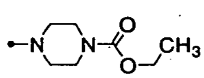
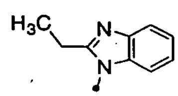
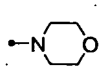
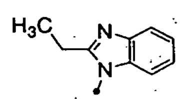
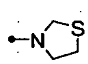
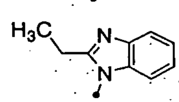
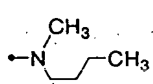
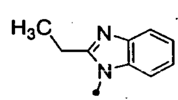
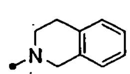
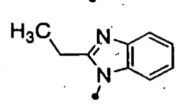
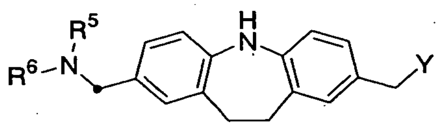
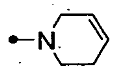
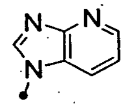
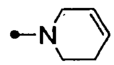
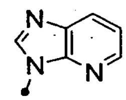
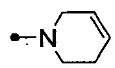
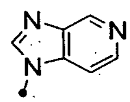
Compound No.	•—NR^5R^6	•—Y	Mass Spectrometric Data
76			MS m/z 466 $(M+H)^+$
77			MS m/z 449 $(M+H)^+$
78			MS m/z 437 $(M+H)^+$
79			MS m/z 479 $(M+H)^+$
80			MS m/z 534 $(M+H)^+$
81			MS m/z 541 $(M+H)^+$
82			MS m/z 542 $(M+H)^+$
83			MS m/z 524 $(M+H)^+$
84			MS m/z 453 $(M+H)^+$
85			MS m/z 455 $(M+H)^+$
86			MS m/z 453 $(M+H)^+$
87			MS m/z 499 $(M+H)^+$

Table 7



Compound No.	•—NR5R6	•—Y
88		
89		
90		

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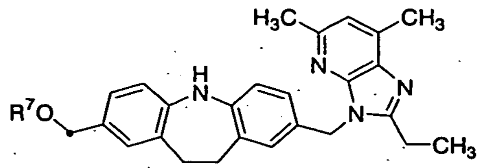
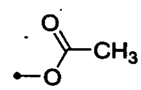
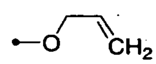
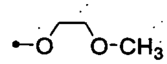
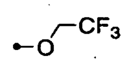
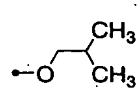
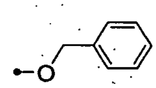
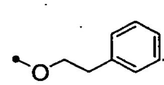
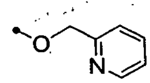
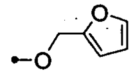


Compound No.	•—OR^7
92	
93	•—OH
94	•—O–CH_3
95	
96	
97	
98	
99	
100	
101	
102	

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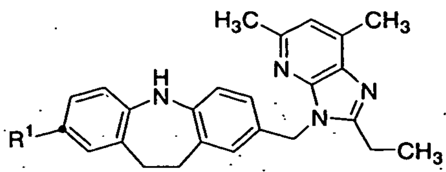


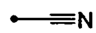
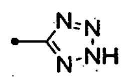
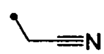
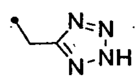
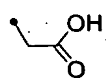
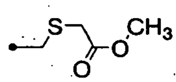
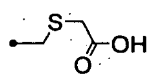
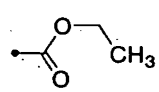
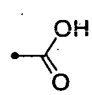
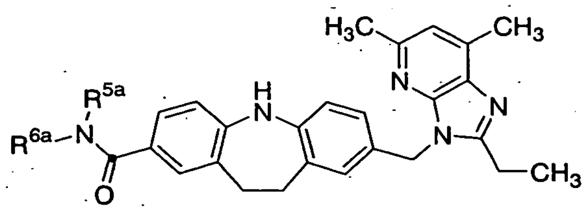
Compound No.	$\bullet$—R^1
103	
104	
105	
106	
107	
108	
109	
110	
111	

Table 10



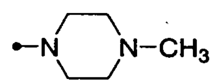
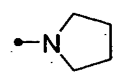
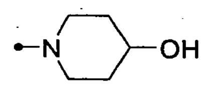
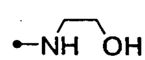
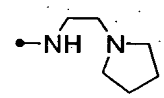
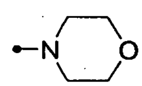
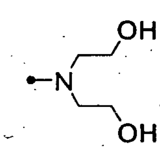
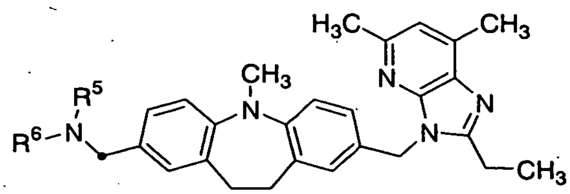
Compound No.	$\bullet\!-NR^{5a}R^{6a}$
112	
113	
114	
115	
116	
117	
118	
119	$\bullet\!-NH_2$

Table 11



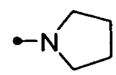
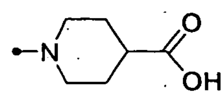
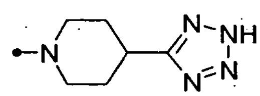
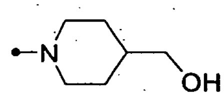
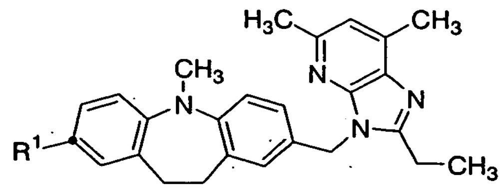
Compound No.	$\bullet-NR^5R^6$
120	
121	
122	
123	

Table 12



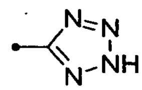
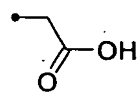
Compound No.	•—R^1
124	
125	

Table 13

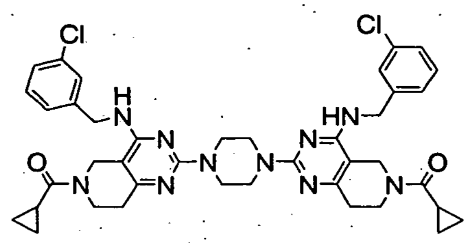
Compound No.	
91	

Table 14

Compound No.	Analytical Data
5	MS m/z 508 $(M+H)^+$
6	MS m/z 563 $(M+H)^+$
7	MS m/z 570 $(M+H)^+$
8	MS m/z 571 $(M+H)^+$
9	MS m/z 553 $(M+H)^+$
10	MS m/z 484 $(M+H)^+$
11	MS m/z 482 $(M+H)^+$
12	MS m/z 528 $(M+H)^+$

Brief Description of the Drawings

Fig. 1 shows a suppressive effect of compound 1 (by intraperitoneal administration) on bronchoconstriction induced by antigen. In Fig. 1, each of the symbols (##, **) has the following meaning, respectively.

##: $p = 0.0043$ (ratio of a positive control group to a negative control group; Student's t-test)

**: $p = 0.0047$ (ratio of a group to which compound 1 was intraperitoneally administered to a positive control group; Student's t-test)

Fig. 2 shows a suppressive effect of compound 1 (by oral administration) on bronchoconstriction induced by antigen. In Fig. 2, each of the symbols (###, *) has the following meaning, respectively.

###: $p < 0.0001$ (ratio of a positive control group to a negative control group; Student's t-test)

*: $p = 0.0248$ (ratio of a group to which compound 1 was orally administered to a positive control group; Student's t-test)

Fig. 3 shows a suppressive effect of compound 1 on airway hyperreactivity induced by antigen. In Fig. 3, the symbol (#) has the following meaning.

#: $p = 0.0182$ (ratio of a positive control group to a negative control group; Student's t-test)

Fig. 4 shows a suppressive effect of compound 1 on

eosinophil infiltration in airway induced by antigen. In Fig. 4, each of the symbols (### , ***) has the following meaning, respectively.

###: $p = 0.0009$ (ratio of a positive control group to a negative control group; Aspin-Welch test)

***: $p = 0.0030$ (ratio of a group to which compound 1 was administered to a positive control group; Student's t-test)

****: $p = 0.0015$ (ratio of a reference group to a positive control group; Student's t-test)

Pharmacological action of the compounds is illustrated by way of Test Examples.

Test Example 1: Antagonistic action on GPR4

Cells for GPR4 assay prepared in Referential Example 61 (said assay cells express GPR4 by stimulation with 17β-estradiol) were seeded on white plates at the rate of 10^5 cells/well, and a solution where 17β-estradiol (manufactured by Sigma) was diluted with a medium so as to make 10 nmol/L in the reaction solution and test compounds were added to the plate and the mixture was incubated at 37°c for 6 hours in a 5% CO_2 incubator. After that, a Steady Glo Luciferase Assay System (manufactured by Promega) solution was added to stop the reaction and the amount of luminescence during one second was measured using a Top Count (Packard, Meriden, CT, U. S. A.).

Activity of the each of test compounds (antagonistic

action) was expressed by an inhibition rate calculated on the basis of counts per second in the presence or absence of 17β-estradiol as shown by the following formula. IC_{50} value was calculated from the inhibition rate by a linear approximate analysis of the Logit-Log conversion method.

In the formula, A, B and C each have the following meaning, respectively.

A: counts per second when 17β-estradiol and the test compound were added

B: counts per second when neither 17β-estradiol nor the test compound was added

C: counts per second when only 17β-estradiol was added

Inhibition Rate (%) = $[1 - \{(A - B)/(C - B)\}] \times 100$

The result is shown in Table 15.

Table 15

Compound Nos.	IC_{50} (nmol/L)
1	4.0
2	3.2
3	2.3
4	5.8
5	14

From the above results, compound (I) has been shown to have an antagonistic action to GPR4.

Test Example 2: Suppressive effect on antigen-induced bronchoconstriction, airway hyperreactivity and eosinophil infiltration in airway

Suspention prepared by mixing of 50 μg of ovalbumin and 1 mg of aluminum hydroxide in a physiological saline solution (Otsuka Physiological Saline; manufactured by Otsuka Pharmaceutical) was intraperitoneally administered to BALB/c male mice (7 weeks age) for two times with an interval of one week to sensitize and, after 14, 18 and 22 days from the final sensitization, 1% solution of ovalbumin in a physiological saline solution or a physiological saline solution (a negative control group) was inhaled for 30 minutes respectively to induce an antigen-antibody reaction. In the case of measurement of bronchoconstriction, compound 1 was suspended in a 0.5% aqueous solution of methylcellulose (solvent) and, 1 hour before inhalation of antigen after 14 days from the final sensitization, 100 mg/kg of the suspention was orally administered or, 5 minutes before that, 30 mg/kg was intraperitoneally administered. In the case of measurement of airway hyperreactivity or eosinophil infiltration in airway, compound 1 was suspended as mentioned above and, after 14, 18 and 22 days from the final sensitization, 100 mg/kg of the suspention was orally administered 1 hour before and 6 hours after inhalation of each antigen. As a reference drug, prednisolone which is a steroid used for the treatment of asthma was orally administered at a dose of 30 mg/kg (as a solution being suspended in 0.5% aqueous solution of methylcellulose) once, 1 hour before inhalation of each antigen after 14, 18 and 22 days from the final sensitization. In a

positive control group, the solvent was administered instead of a suspension of a test compound. (In the case of measurement of bronchoconstriction, the solvent was administered once, 1 hour before inhalation of antigen after 14 days from the final sensitization as for oral administration, or the solvent was administered once 30 minutes before inhalation of antigen after 14 days from the final sensitization as for intraperitoneal administration. In the case of measurement of airway hyperreactivity and eosinophil infiltration in airway, the solvent was administered 1 hour before and 6 hours after inhalation of each antigen after 14, 18 and 22 days from the final sensitization.)

With regard to the bronchoconstriction, airway resistance (penh) was evaluated by a measuring apparatus for respiration function of mice (BioSystems XA; Buxco Electronics, Inc., Sharon, CT, U. S. A.) for 30 minutes from immediately after inhalation of antigen after 14 days from the final sensitization and evaluation was conducted by calculating the area under curve during the 30 minutes ($AUC_{0-30\ min}$). Further, after 24 hours from final inhalation of antigen, airway hyperreactivity and eosinophil infiltration in bronchoalveolar lavage fluid were evaluated.

With regard to an airway hyperreactivity test, bronchoconstriction after inhalation of 1.5 to 25 mg/ml of methacholine for 3 minutes (inhaled after 24 hours from 22 days

after the final sensitization) was determined by a measuring apparatus for respiration function of mice (BioSystems XA; Buxco Electronics, Inc., Sharon, CT, U. S. A.) and evaluation was conducted by calculating the area under curve (AUC) from a curve of dose of methacholine vs. bronchoconstriction. With regard to eosinophil filtration, total cell numbers in the bronchoalveolar lavage fluid were measured by an automatic measuring apparatus for blood cell count (Celltac α MEK-6158; Nippon Koden, Tokyo), then the smears were prepared by Cytospin 3 (Shandon, Inc., Pittsburgh, PA, U. S. A.) and evaluation was conducted by a morphological classification under a microscope. Eosinophil numbers were calculated by multiplying the total cell numbers by percentage of eosinophil cells. The test was conducted for a group comprising ten mice.

Result for bronchoconstriction is shown in Fig. 1 (by intraperitoneal administration) and in Fig. 2 (by oral administration), result for airway hyperreactivity is shown in Fig. 3 and result for eosinophil infiltration in airway is shown in Fig. 4.

As shown in Fig. 1, $AUC_{0-30min}$ of the bronchoconstriction in the positive control group (18.22 ± 1.02; mean value ± standard error) significantly increased as compared with $AUC_{0-30min}$ of the negative control group (14.77 ± 0.27) ($p = 0.0043$, Student's t-test). $AUC_{0-30min}$ of the group to which compound 1 was intraperitoneally administered was 14.60 ± 0.46 and, as compared

with the positive control group, the bronchoconstriction was significantly suppressed by 105% ($p = 0.0047$, Student's t-test).

As shown in Fig. 2, $AUC_{0\text{-}30min}$ of the bronchoconstriction in the positive control group (19.61 ± 0.75, mean value ± standard error) significantly increased as compared with $AUC_{0\text{-}30min}$ of the negative control group (13.37 ± 0.20) ($p < 0.0001$, Student's t-test). $AUC_{0\text{-}30min}$ of the group to which compound 1 was orally administered was 16.85 ± 0.84 and, as compared with the positive control group, the bronchoconstriction was significantly suppressed by 44% ($p = 0.0248$, Student's t-test).

As shown in Fig. 3, AUC of airway hyperreactivity of the positive control group (335.13 ± 52.6, mean value ± standard error) significantly increased as compared with AUC of the negative control group (184.7 ± 27.5) ($p = 0.0182$, Student's t-test). AUC of the group to which compound 1 was administered was 243.23 ± 48.7 and, as compared with the positive control group, airway hyperreactivity was suppressed by 60%. AUC of the group to which prednisolone was administered was 269.12 ± 46.7 and, as compared with the positive control group, airway hyperreactivity was suppressed by 43%.

As shown in Fig. 4, eosinophil numbers in bronchoalveolar lavage fluid of the negative control group was $0.00 \pm 0.00 \times 10^5$ per mouse and, in the positive control group, a significant increase of $2.77 \pm 0.46 \times 10^5$ was noted ($p = 0.0009$, Aspin-Welch test). Eosinophil numbers in the group to which compound 1

was administered and the group to which prednisolone was administered per mouse were $0.92 \pm 0.26 \times 10^5$ and $0.76 \pm 0.25 \times 10^5$, respectively. As compared with the positive control group, eosinophil numbers were significantly decreased by 67% in the group to which compound 1 was administered ($p = 0.0030$, Student's t-test). In the group to which prednisolone was administered, eosinophil numbers were significantly decreased by 73% ($p = 0.0015$, Student's t-test).

From the above results, it has been suggested that a substance capable of suppressing the function involved in signal transduction of protein comprising an amino acid sequence represented by SEQ ID NO: 11 is useful as an agent for treatment of itching.

The medicament of the present invention is characterized in containing a substance selected from a group consisting of the nitrogen-containing tricyclic compound represented by the formula (I) or the quaternary ammonium salt thereof, or the pharmaceutically acceptable salt thereof and the hydrate and the solvate thereof as an active ingredient. With regard to the medicament of the present invention, the above-mentioned substance which is an active ingredient may be administered as it is but, usually, it is desirable to administer in a form of a pharmaceutical composition containing the above-mentioned substance which is an active ingredient and one or more additives for the preparation. Such a pharmaceutical composition is able

to be prepared by a method which is known or common in the field of pharmaceutical preparation science. The medicament according to the present invention in a form of a pharmaceutical composition may contain one or more other pharmaceutically active ingredient(s). Incidentally, the pharmaceutical of the present invention is able to be applied to mammals including a human being.

With regard to the administration route of the medicament of the present invention, there is no particular limitation and the most effective administration route for the treatment and/or the prevention may be appropriately selected from oral administration and parenteral administration such as intravenous injection. An example of the pharmaceutical preparation suitable for oral administration is tablets or the like and an example of the pharmaceutical preparation suitable for parenteral administration is injection preparation or the like.

In the manufacture of a solid preparation such as tablet, it is possible to use excipient such as lactose and mannitol; disintegrating agent such as starch; lubricant such as magnesium stearate; binder such as hydroxypropyl cellulose; surfactant such as fatty acid ester; plasticizer such as glycerol or the like.

Among the preparation suitable for parenteral administration, preparation for administration into blood

vessel such as injection preparation may be preferably prepared using an aqueous medium which is isotonic to human blood. For example, an injection preparation is able to be prepared according to a conventional method as solution, suspension or dispersion together with an appropriate excipient using an aqueous medium selected from salt solution, glucose solution, a mixture of salt and glucose solution, etc. For the manufacture of pharmaceutical preparation for parenteral use, it is possible to use one or more additive(s) for pharmaceutical preparations selected from diluent, flavor, antiseptic, excipient, disintegrating agent, lubricant, binder, surfactant, plasticizer, and the like.

There is no particular limitation for the dose and administering frequency of the pharmaceutical of the present invention but it is possible to appropriately select depending upon various conditions such as type of the above substance which is an active ingredient, administration route, object of treatment and/or prevention, age and body weight of patient, nature of symptom and degree of severeness. For example, it is preferred to administer 0.1 to 100 mg/kg per day for an adult by dividing into three to four times a day. However, dose and administering frequency as such may vary depending upon the above-mentioned various conditions, etc.

Best Mode for Carrying Out the Invention

The present invention is now more specifically illustrated by way of the following Referential Examples and Examples, the scope of the present invention is not limited by these Examples, or the like.

Physicochemical data for each of the compounds in the following Referential Examples were measured by the following instruments.

^{1}H-NMR: JOEL JNM-EX270 (270 MHz) or JEOL JNM-GX270 (270 MHz)

MS: Micromass LCT or Micromass Quatro (measured by an APCI method)

Referential Example 1: Synthesis of compound 1 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-(4-methylpiperazin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine (30.0 g, 78.4 mmol) mentioned in the JP-A-7-61983 was dissolved in a mixed solvent of chloroform (300 mL) and acetic acid (300 mL), then 1-methylpiperazine (23.6 g, 236 mmol) and formaldehyde (37% aqueous solution; 7.64 g, 94.1 mmol) were added thereto and the mixture was heated at 60°C and stirred for 18 hours. After confirming the progress of the reaction by a thin-layer chromatography, a saturated aqueous solution of sodium bicarbonate was added under cooling with ice and extracted with

ethyl acetate. The organic layer was successively washed with a saturated aqueous solution of sodium bicarbonate, water and a saturated aqueous solution of salt, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crystals separated therefrom were triturated with ethyl acetate to give compound 1 (27.4 g, 55.4 mmol, yield: 71%).

APCI-MS: m/z 495 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.6 Hz, 3H), 2.27 (s, 3H), 2.45 (m, 8H), 2.60 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.6 Hz, 2H), 2.98 (m, 4H), 3.38 (s, 2H), 5.34 (s, 2H), 6.00 (s, 1H), 6.57-6.66 (m, 2H), 6.79-7.00 (m, 5H).

The corresponding fumarate was prepared according to the following process.

Thus, the above compound 1 (15 g) was dissolved in methanol (110 mL) and fumaric acid (7.0 g, 2.0 equivalents) was added. A suspension wherefrom crystals were separated was once concentrated to dryness, acetonitrile (100 mL) was added and a suspension was stirred for not shorter than 1 hour. After that, the crystals were filtered and dried *in vacuo* to give a difumarate of compound 1 (20.1 g, yield: 91%).

Referential Example 2: Synthesis of compound 2 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-(1,2,5,6-tetrahydropyridin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

The operation similar to that in Referential Example 1

was conducted using 1,2,3,6-tetrahydropyridine instead of 1-methylpiperazine and, starting from 2-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine mentioned in the JP-A-7-61983, compound 2 was obtained in the yield of 20%.

APCI-MS: m/z 478 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 2.04 (m, 2H), 2.53 (t, J = 5.7 Hz, 2H), 2.60 (s, 3H), 2.62 (s, 3H), 2.79 (q, J = 7.5 Hz, 2H), 2.86-3.02 (m, 6H), 3.45 (s, 2H), 5.33 (s, 2H), 5.64 (m, 1H), 5.74 (m, 1H), 6.02 (s, 1H), 6.57-6.70 (m, 2H), 6.78-6.82 (m, 2H), 6.88 (s, 1H), 6.95-7.00 (m, 2H).

Referential Example 3: Synthesis of compound 3 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-(pyrrolidin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

The operation similar to that in Referential Example 1 was conducted using pyrrolidine instead of 1-methylpiperazine and, starting from 2-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine mentioned in the JP-A-7-61983, compound 3 was obtained in the yield of 20%.

APCI-MS: m/z 466 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 1.78 (m, 4H), 2.50 (m, 4H), 2.60 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.5 Hz, 2H), 2.98 (m, 4H), 3.50 (s, 2H), 5.34 (s, 2H), 6.02

(s, 1H), 6.58-6.66 (m, 2H), 6.79-6.81 (m, 2H), 6.88 (s, 1H), 6.98-7.02 (m, 2H).

Referential Example 4: Synthesis of compound 4 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-morpholinomethyl-10,11-dihydro-5H-dibenz[b,f]azepine}

The operation similar to that in Referential Example 1 was conducted using morpholine instead of 1-methylpiperazine and, starting from 2-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine mentioned in the JP-A-7-61983, compound 4 was obtained in the yield of 46%.

APCI-MS: m/z 482 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 2.43 (m, 4H), 2.60 (m, 3H), 2.63 (m, 3H), 2.79 (q, J = 7.5 Hz, 2H), 2.98 (m, 4H), 3.38 (s, 2H), 3.69 (m, 4H), 5.34 (s, 2H), 6.07 (s, 1H), 6.58-6.67 (m, 2H), 6.78-6.81 (m, 2H), 6.88 (s, 1H), 6.96-7.01 (m, 2H).

Referential Example 5: Synthesis of compound 5 to compound 12

2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine (19 mg, 0.050 mmol) mentioned in the JP-A-7-61983 was dissolved in a mixed solvent of chloroform (0.30 mL) and acetic acid (0.30 mL), then a chloroform solution of the corresponding R^5R^6NH (1.0 mol/L,

0.15 mL) and formaldehyde (37% aqueous solution, 0.005 mL) were added thereto and the mixture was heated at 60°C and stirred for 20 hours. After confirming the progress of the reaction by a thin-layer chromatography, the solvent was evaporated and the residue was dissolved in chloroform and washed with water for two times. The organic layer was dried over anhydrous sodium sulfate and concentrated. To the residue were added chloroform (0.50 mL) and N-methylisatoic anhydride polystyrene (manufactured by Nova Biochem, 0.15 mL) were added followed by stirring at room temperature for one night. The resin in the reaction mixture was filtered off and the residue was purified by means of ion-exchange chromatography (Bondecil SCX, manufactured by Varian, eluted with a 2 mol/L methanolic solution of ammonia) to give objective compound 5 to compound 12.

Structures and analytical data (APCI-MS) of the compounds are shown in Table 1 and Table 14, respectively.

Referential Example 6: Synthesis of compound 13 {1-[8-(2-Ethyl-5,7-dimethyl-3H-imidazol[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine-2-ylmethyl]-1-methyl-pyrrolidinium iodide}

Compound 3 (11.4 g, 24.5 mmol) prepared in Referential Example 3 was dissolved in dichloromethane (200 mL), methyl iodide (1.98 mL, 31.8 mmol) was added thereto and the mixture was stirred at room temperature for 10 hours. After the reaction solution was concentrated *in vacuo*, ethyl acetate was added

thereto. The resulting suspension was heated at 60°C, stirred for 0.5 hour and then stirred at room temperature for 1 hour. The solid separated out was filtered to give compound 13 (13.7 g, 22.5 mmol, yield: 92%).

APCI-MS: m/z 480 ($[M - I]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.31 (t, J = 7.6 Hz, 3H), 2.13 (br s, 2H), 2.25 (br s, 2H), 2.58 (s, 3H), 2.62 (s, 2H), 2.79 (q, J = 7.6 Hz, 2H), 2.85 (m, 4H), 3.06 (s, 3H), 3.52 (br s, 2H), 3.83 (br s, 2H), 4.74 (s, 2H), 5.32 (s, 2H), 6.76 (m, 2H), 6.88 (s, 1H), 6.95-7.18 (m, 4H), 7.43 (s, 1H).

Referential Example 7: Synthesis of compound 14 {2-(2,5-Dihydropyrrol-1-ylmethyl)-8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenzo[b,f]azepine}

The operation similar to that in Referential Example 1 was conducted using 2,5-dihydropyrrole instead of 1-methylpiperazine and, starting from 2-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine mentioned in the JP-A-7-61983, compound 14 was obtained in the yield of 82%.

APCI-MS: m/z 464 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 2.59 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.5 Hz, 2H), 2.9-3.1 (m, 4H), 3.45 (s, 4H), 3.70 (s, 2H), 5.34 (s, 2H), 5.87 (s, 2H), 6.07 (s, 1H), 6.59 (d, J = 8.7 Hz , 2H), 6.63 (d, J = 8.7 Hz , 2H),

6.75-6.85 (m, 2H), 6.88 (s, 1H), 7.00-7.05 (m, 2H).

Referential Example 8: Synthesis of compound 15 <Methyl{N-8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine-2-ylmethyl}-N-methylamino}acetate>

The operation similar to that in Referential Example 1 was conducted using sarcosine methyl ester hydrochloride instead of 1-methylpiperazine and, starting from 2-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine mentioned in the JP-A-7-61983, compound 15 was obtained in the yield of 31%.

APCI-MS: m/z 498 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.6 Hz, 3H), 2.36 (s, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.6 Hz, 2H), 2.98 (m, 4H), 3.23 (s, 2H), 3.53 (s, 2H), 3.70 (s, 3H), 5.34 (s, 2H), 5.98 (s, 1H), 6.59-6.67 (m, 2H), 6.82 (m, 2H), 6.88 (s, 1H), 6.97-7.02 (m, 2H).

Referential Example 9: Synthesis of compound 16 {Ethyl 1-[8-2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine-2-ylmethyl] piperidine-4-carboxylate}

The operation similar to that in Referential Example 1 was conducted using ethyl isonipecotate instead of 1-methylpiperazine and, starting from 2-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-

ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine mentioned in the JP-A-7-61983, compound 16 was obtained in the yield of 60%.

APCI-MS: m/z 552 ([M + H]$^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.23 (t, J = 7.0 Hz, 3H), 1.30 (t, J = 7.6 Hz, 3H), 1.68-1.90 (m, 6H), 1.97 (td, J = 11.3, 2.7 Hz, 2H), 2.26 (m, 1H), 2.60 (s, 3H), 2.62 (s, 3H), 2.79 (q, J = 7.6 Hz, 2H), 2.83 (m, 2H), 2.98 (m, 4H), 3.36 (s, 2H), 4.11 (q, J = 7.0 Hz, 2H), 5.33 (s, 2H), 6.03 (s, 1H), 6.57-6.66 (m, 2H), 6.78-6.82 (m, 2H), 6.88 (s, 1H), 6.94-6.99 (m, 2H).

Referential Example 10: Synthesis of compound 17 <2-{N-[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine-2-ylmethyl]-N-methylamino}ethanol>

Lithium aluminum hydride (15.7 mg, 0.38 mmol) was suspended in tetrahydrofuran (0.3 mL) with stirring under cooling with ice. Compound 15 (126 mg, 0.253 mmol) prepared in Referential Example 8 dissolved in tetrahydrofuran (0.9 mL) was added thereto followed by stirring at room temperature for 1.5 hours. After confirming the progress of the reaction by means of thin-layer chromatography, water (0.016 mL), a 2 mol/L aqueous solution of sodium hydroxide (0.016 mL) and water (0.048 mL) were dropped thereinto successively with stirring. The precipitate was filtered off, the filtrate was concentrated and the resulting residue was purified by an NH-silica gel chromatography (eluting solvent: ethyl acetate) to give

compound 17 (47.6 mg, 0.101 mmol, yield: 40%).

APCI-MS: m/z 470 ([M + H]$^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 1.7 (br s, 1H), 2.21 (s, 3H), 2.57 (t, J = 5.5 Hz, 2H), 2.60 (s, 3H), 2.63 (s, 3H), 2.80 (q, J = 7.5 Hz, 2H), 2.98 (m, 4H), 3.44 (s, 2H), 3.61 (t, J = 5.5 Hz, 2H), 5.34 (s, 2H), 5.99 (s, 1H), 6.59-6.67 (m, 2H), 6.81 (m, 2H), 6.88 (s, 1H), 6.91-6.98 (m, 2H).

Referential Example 11: Synthesis of compound 18 <{1-[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl] piperidin-4-yl}methanol>

Compound 16 was used instead of compound 15 and, in the manner similar to that in Referential Example 10, compound 18 was prepared in a yield of 50%.

APCI-MS: m/z 510 ([M + H]$^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.6 Hz, 3H), 1.24-1.74 (m, 6H), 1.91 (m, 2H), 2.60 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.6 Hz, 2H), 2.86-3.02 (m, 6H), 3.37 (s, 2H), 3.48 (d, J = 6.3 Hz, 2H), 5.34 (s, 2H), 5.98 (s, 1H), 6.58-6.67 (m, 2H), 6.82 (m, 2H), 6.89 (s, 1H), 6.94-7.00 (m, 2H).

Referential Example 12: Synthesis of compound 19 <{N-[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]-N-methylamino}acetic acid>

Compound 15 (151 mg, 0.303 mmol) prepared in Referential

Example 8 was dissolved in methanol (3.0 mL), a 1 mol/L methanol solution of sodium hydroxide (1.5 mL) was added thereto and the mixture was heated at 60°C and stirred for 9 hours. After confirming the progress of the reaction by a thin-layer chromatography, it was cooled to room temperature and pH was adjusted to 6.0 by addition of 4 mol/L hydrochloric acid. The crystals separated out therefrom were filtered and dried *in vacuo*. The crystals were suspended in ethyl ether, stirred for 1 hour under heating with refluxing and then stirred for 1 hour at room temperature. The crystals were filtered and dried *in vacuo* to give compound 19 (119 mg, 0.246 mmol, yield: 81%).

APCI-MS: m/z 483 ($[M + H]^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.23 (t, J = 7.4 Hz, 3H), 2.34 (s, 3H), 2.48-2.52 (s x 2, 6H, overlapped with DMSO), 2.78 (q, J = 7.4 Hz, 2H), 2.89 (m, 4H), 3.11 (s, 2H), 3.66 (s, 2H), 5.29 (s, 2H), 6.75-7.02 (m, 7H), 8.36 (s, 1H).

Referential Example 13: Synthesis of compound 20 {1-[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl] piperidine-4-carboxylic acid}

The operation similar to that in Referential Example 12 was conducted using compound 16 instead of compound 15 to give compound 20 in a yield of 70%.

APCI-MS: m/z 524 ($[M + H]^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.23 (t, J = 7.4 Hz, 3H), 1.52 (m, 2H), 1.75 (m, 2H), 1.97 (m, 2H), 2.18 (m, 1H), 2.48-2.54 (s x 2, 6H, overlapped with DMSO), 2.71-2.92(m, 8H), 3.32 (s, 2H), 5.29 (s, 2H), 6.75-6.94 (m, 7H), 8.23 (s, 1H).

Referential Example 14: Synthesis of compound 21 <{N-[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-yl methyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]-N-methylamino}acetonitrile>

Compound 13 (700 mg, 1.15 mmol) prepared in Referential Example 6 was dissolved in chloroform (1.2 mL), then methylaminoacetonitrile (368 mg, 3.46 mmol) and triethylamine (0.561 mL, 4.03 mmol) were added thereto and the mixture was stirred for one night under refluxing. The reaction solution was cooled to room temperature, a saturated aqueous solution of sodium bicarbonate was added and the mixture was extracted with chloroform for three times. The organic layers were combined, washed with a saturated aqueous saline solution, dried over anhydrous magnesium sulfate and concentrated and the residue was purified by a silica gel chromatography (eluting solvent: methanol/chloroform = 1/99). Ethanol was added to the concentrated residue of the fraction containing the objective substance and the resulting suspension was stirred at 60°C for 0.5 hour and then stirred at room temperature for 1 hour. The crystals separated out therefrom were filtered and dried *in vacuo* to give compound 21 (415 mg, 0.893 mmol,

yield: 78%).

APCI-MS: m/z 465 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 2.42 (s, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.5 Hz, 2H), 2.98 (m, 4H), 3.43 (s, 2H), 3.48 (s, 2H), 5.34 (s, 2H), 6.10 (s, 1H), 6.58-6.69 (m, 2H), 6.78-6.83 (m, 2H), 6.88 (s, 1H), 6.95-7.02 (m, 2H).

Referential Example 15: Synthesis of compound 22 {N-[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]-N-[2-(pyrrolidin-1-yl)ethyl]amine difumarate}

Step 1

Compound 93 (1.25 g, 3.03 mmol) prepared in Referential Example 24 which is mentioned later was dissolved in a mixed solvent of chloroform (54 mL) and acetone (6 mL), then manganese dioxide (2.7 g, 31 mmol) was added thereto and the mixture was stirred for one night at room temperature. After confirming the progress of the reaction by a thin-layer chromatography, the solid was filtered off using a Celite and the filtrate was concentrated. Ethyl acetate was added to the residue, the resulting suspension was stirred for 0.5 hour under reflux, then cooled to room temperature and stirred for 0.5 hour. The crystals separated out therefrom were filtered and dried *in vacuo* to give 8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-

ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-carboaldehyde (1.02 g, 2.48 mmol, yield: 82%).

APCI-MS: m/z 411 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.31 (t, J = 7.5 Hz, 3H), 2.60 (s, 3H), 2.64 (s, 3H), 2.80 (q, J = 7.5 Hz, 2H), 2.99 (m, 2H), 3.06 (m, 2H), 5.37 (s, 2H), 6.60-6.91 (m, 6H), 7.52-7.61 (m, 2H), 9.77 (s, 1H).

Step 2

8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-carboaldehyde (0.300 g, 0.73 mmol) prepared in step 1 was suspended in a mixed solvent of tetrahydrofuran (10 mL) and chloroform (6 mL), then 2-(pyrrolidin-1-yl)ethylamine (139 μL, 1.10 mmol) was added thereto and the mixture was heated to reflux for 10 minutes. After that, the reaction solution was cooled to room temperature, sodium triacetoxyborohydride (464 mg, 2.19 mmol) was added thereto and the mixture was stirred for 12 hours at room temperature. To the reaction solution were added ethyl acetate and a 1 mol/L aqueous solution of sodium hydroxide and the organic layer was dried over anhydrous magnesium sulfate. After that, the solution was concentrated *in vacuo* and the residue was purified by a silica gel chromatography (eluting solvent: chloroform/2 mol/L methanolic solution of ammonia = 20/1) to give N-[8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-

ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]-N-[2-(pyrrolidin-1-yl)ethyl]amine (0.301 g, 0.592 mmol, yield: 81%). This was made into a fumarate by the method similar to Referential Example 1 to give compound 22.

APCI-MS: m/z 509 ($[M + H]^+$)

^1H NMR ($DMSO-d_6$) δ(ppm): 1.23 (t, J = 7.4 Hz, 3H), 1.65-1.85 (m, 4H), 2.50 (s, 3H), 2.51 (s, 3H), 2.6-2.7 (m, 4H), 2.7-3.0 (m, 8H), 3.86 (s, 2H), 5.29 (s, 2H), 6.55 (s, 4H), 6.75-6.95 (m, 6H), 7.0-7.15 (m, 2H), 8.43 (s, 1H).

Referential Example 16: Synthesis of compound 23 {N-[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]-N-(2-methoxyethyl) amine monofumarate}

The operation similar to that in step 2 of Referential Example 15 was conducted using 2-methoxyethylamine instead of 2-(pyrrolidin-1-yl)ethylamine to give N-[8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]-N-(2-methoxyethyl) amine in a yield of 78%. This was converted to a fumarate by the manner similar to that in Referential Example 1 to give compound 23.

APCI-MS: m/z 470 ($[M + H]^+$)

^1H NMR ($DMSO-d_6$) δ(ppm): 1.23 (t, J = 7.4 Hz, 3H), 2.50 (s, 3H), 2.51 (s, 3H), 2.80 (q, J = 7.4 Hz, 2H), 2.8-3.0 (m, 6H), 3.24 (s, 3H), 3.49 (t, J = 6.5 Hz, 2H), 3.80 (s, 2H), 5.29

(s, 2H), 6.48 (s, 2H), 6.84 (d, J = 8.1 Hz, 1H), 6.85-7.0 (m, 4H), 7.0-7.1 (m, 2H), 8.43 (s, 1H).

Referential Example 17: Synthesis of compound 24 <2-{[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]} aminoethanol 0.5 fumarate}

The operation similar to that in step 2 of Referential Example 15 was conducted using 2-ethanolamine instead of 2-(pyrrolidin-1-yl)ethylamine to give 2-{[8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl] amino}ethanol in a yield of 39%.

This was converted to a fumarate by the manner similar to that in Referential Example 1 to give compound 24.

APCI-MS: m/z 456 ($[M + H]^+$)

^1H NMR ($DMSO-d_6$) δ(ppm): 1.23 (t, J = 7.4 Hz, 3H), 2.50 (s, 3H), 2.51 (s, 3H), 2.70-2.75 (m, 2H), 2.77 (q, J = 7.4 Hz, 2H), 2.85-2.9 (m, 4H), 3.55 (t, J = 5.5 Hz, 2H), 3.78 (s, 2H), 5.29 (s, 2H), 6.44 (s, 1H), 6.79 (dd, J = 1.5 Hz, 8.3 Hz, 1H), 6.85-6.95 (m, 4H), 7.0-7.1 (m, 2H), 8.39 (s, 1H).

Referential Example 18: Synthesis of compound 25 <{[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-yl]methyl} amine monofumarate>

Compound 13 (0.300 g, 0.516 mmol) prepared in Referential

Example 6 was dissolved in a 7 mol/L methanolic solution of ammonia (5 mL), sealed and heated at 80°C for 48 hours. After that, the reaction solution was concentrated *in vacuo*. The residue was purified by a silica gel chromatography (eluting solvent: chloroform/2mol/L methanolic ammonia solution = 20/1) to give {[8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-yl]methyl} amine (0.135 g, 0.219 mol, yield: 64%).

This was converted to a fumarate by the manner similar to that in Referential Example 1 to give compound 25.

APCI-MS: m/z 412 ($[M + H]^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.23 (t, J = 7.4 Hz, 3H), 2.50 (s, 3H), 2.51 (s, 3H), 2.77 (q, J = 7.4 Hz, 2H), 2.85-2.9 (m, 4H), 3.81 (s, 2H), 5.29 (s, 2H), 6.42 (s, 2H), 6.8-7.0 (m, 5H), 7.0-7.15 (m, 2H), 8.46 (s, 1H).

Referential Example 19: Synthesis of compound 26 {N-[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]-N-methyl-N-(2H-tetrazol-5-ylmethyl)amine}

Compound 13 (667 mg, 1.10 mmol) prepared in Referential Example 6 was dissolved in chloroform (11 mL), then N-methyl-N-(2-trityl-2H-tetrazol-5-ylmethyl)amine (390 mg, 1.10 mmol) prepared in Referential Example 22 and triethylamine (0.31 mL, 2.3 mmol) were added thereto and the mixture was stirred

at 60°C for one night. The reaction solution was cooled to room temperature, a saturated aqueous solution of sodium bicarbonate was added and the mixture was extracted with chloroform for three times. The organic layers were combined, washed with a saturated saline solution, dried over anhydrous magnesium sulfate and concentrated. The residue was passed through silica gel (eluting solvent: methanol/chloroform = 2/98) to remove starting point components and concentrated. To the residue were added acetone (1.9 mL), water (1.9 mL) and acetic acid (1.9 mL) and the mixture was stirred at 60°C for 1.5 hours. The reaction solution was cooled down to 0°C, separated substances were filtered off, the filtrate was concentrated and the resulting residue was recrystallized from ethanol to give compound 26 (66.7 mg, 0.131 mmol, yield: 12%).

APCI-MS: m/z 508 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.32 (t, J = 5.0 Hz, 3H), 2.58 (s, 3H), 2.63 (s, 3H), 2.75-2.79 (m, 7H), 2.81 (q, J = 5.0 Hz, 2H), 4.08 (s, 2H), 4.28 (s, 2H), 5.34 (s, 2H), 6.37 (s, 1H), 6.46 (d, J = 8.1 Hz, 1H), 6.58 (d, J = 8.1 Hz, 1H), 6.72-6.80 (m, 2H), 6.84-6.94 (m, 3H).

Referential Example 20: Synthesis of compound 27 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-[4-(2H-tetrazol-5-yl)piperidin-1-ylmethyl]-10,11-dihydro-5H-dibenz[b,f]azepine}

The operation similar to that in Referential Example 14

was conducted using piperidine-4-carbonitrile instead of methylaminoacetonitrile to give 1-[8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]-piperidine-4-carbonitrile.

The resulting 1-[8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]-piperidine-4-carbonitrile (0.252 g, 0.500 mmol) was dissolved in toluene (4 mL), then trimethylsilyl azide (0.13 mL, 1.00 mmol) and dibutyltin oxide (12.4 mg, 0.05 mmol) were added and the mixture was heated with stirring at 110°C for 22 hours. The reaction solution was concentrated *in vacuo* and ethanol was added to the residue. The resulting suspension was heated to reflux for 0.5 hour and the solid was filtered to give compound 27 (0.110 g, 0.200 mmol, yield: 40%).

APCI-MS: m/z 548 ($[M + H]^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.22 (t, J = 7.4 Hz, 3H), 1.65-1.85 (m, 2H), 1.9-2.05 (m, 2H), 2.2-2.35 (m, 2H), 2.48 (s, 3H), 2.58 (s, 3H), 2.77 (q, J = 7.4 Hz, 2H), 2.85-3.05 (m, 7H), 3.52 (s, 2H), 5.29 (s, 2H), 6.85-7.05 (m, 8H), 8.36 (s, 1H).

Referential Example 21: Synthesis of compounds 28 to 90

Step 1

1-(10,11-Dihydro-5H-dibenz[b,f]azepin-2-ylmethyl)-1-methylpiperidinium iodide (0.015 g, 0.050 mmol) was dissolved

in dimethyl formamide (0.50 mL), then a chloroform solution of the corresponding YH (wherein Y has the same meaning as defined above) (1.0 mmol/L, 0.060 mL) and lithium hydroxide monohydrate (0.070 g) were added and the mixture was stirred at room temperature for 20 hours. After confirming the progress of the reaction by a thin-layer chromatography, the solvent is evaporated, the residue was dissolved in dichloromethane and the resulting solution was washed with water for three times. The organic layer was dried over anhydrous sodium sulfate and concentrated, chloroform (0.60 mL) and N-methylisatoic anhydride polystyrene (manufactured by Nova Biochem, 0.15 mL) were added thereto and the mixture was stirred at room temperature for one night. The resin in the reaction mixture was filtered off, the filtrate was concentrated and the residue was purified by an ion-exchange chromatography (Bondecil SCX, manufactured by Barian, eluted with 2 mol/L methanolic solution of ammonia) to give various intermediates corresponding to compound (IV) in the Producing Process 1.

Step 2

The operation similar to that in Referential Example 5 was conducted to give desired compounds 28 to 90 from various intermediates corresponding to compound (IV) in the Producing Process 1 obtained in step 1 and the corresponding R^5R^6NH (wherein R^5 and R^6 each have the same meaning as defined above, respectively). Incidentally, compounds 41, 42, 48 and 89 were

isolated as oxalates.

Structures and analytical data (APCI-MS) of compounds 28 to 87 are shown in Tables 2 to 6. Analytical data (^{1}H-NMR) of compounds 29, 30, 36, 41, 42, 48, 53, 54, 60, 65, 66, 72, 77, 78 and 84 are shown below.

Compound 29:

{2-(Benzimidazol-1-ylmethyl)-8-(1,2,5,6-tetrahydropyridin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

^{1}H NMR (DMSO-d_6) δ(ppm): 2.0-2.1 (m, 2H), 2.44 (t, J = 5.6 Hz, 2H), 2.75-2.85 (m, 2H), 2.9-3.0 (m, 4H), 3.32 (s, 2H), 5.31 (s, 2H), 5.5-5.8 (m, 2H), 6.8-7.1 (m, 6H), 7.1-7.3 (m, 2H), 7.56 (d, J = 7.1 Hz, 1H), 7.62 (d, J = 7.4 Hz, 1H), 8.28 (s, 1H), 8.35 (s, 1H).

Compound 30:

{2-(Benzimidazol-1-ylmethyl)-8-(pyrrolidin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

^{1}H NMR (DMSO-d_6) δ(ppm): 1.5-1.7 (m, 4H), 2.3-2.5 (m, 4H), 2.8-3.0 (m, 4H), 3.39 (s, 2H), 5.31 (s, 2H), 6.8-6.95 (m, 4H), 6.95-7.0 (m, 2H), 7.1-7.3 (m, 2H), 7.55 (d, J = 8.9 Hz, 1H), 7.63 (d, J = 8.4 Hz, 1H), 8.26 (s, 1H), 8.34 (s, 1H).

Compound 36:

{2-(Benzimidazo-1-ylmethyl)-8-morpholinomethyl-10,11-dihydro-5H-dibenz[b,f]azepine}

^{1}H NMR (DMSO-d_6)δ(ppm): 2.2-2.4 (m, 4H), 2.8-3.0 (m, 4H), 3.27 (s, 2H), 3.5-3.6 (m, 4H), 5.30 (s, 2H), 6.7-7.1 (m, 6H),

7.1-7.25 (m, 2H), 7.54 (d, J = 7.6 Hz, 1H), 7.62(d, J = 7.6 Hz, 1H), 8.28 (s, 1H), 8.34 (s, 1H).

Compound 41:

{2-(2-Phenylbenzimidazol-1-ylmethyl)-8-(1,2,5,6-tetrahydropyridin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine monooxalate}

^{1}H NMR (DMSO-d_6) δ(ppm): 2.2-2.5 (m, 2H), 2.7-3.0 (m, 4H), 3.0-3.2 (m, 2H), 3.4-3.6 (m, 2H), 4.05 (s, 2H), 5.45 (s, 2H), 5.69 (m, 1H), 5.85 (m, 1H), 6.6-6.8 (m, 2H), 6.88 (d, J = 8.3 Hz, 1H), 6.97 (d, J = 7.9 Hz, 1H), 7.05-7.2 (m, 2H), 7.2-7.5 (m, 2H), 7.5-7.7 (m, 4H), 7.7-7.85 (m, 3H), 8.54 (s, 1H).

Compound 42:

{2-(2-Phenylbenzimidazol-1-ylmethyl)-8-(pyrrolidin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine monooxalate}

^{1}H NMR (DMSO-d_6) δ(ppm): 1.8-2.0 (m, 4H), 2.8-3.0 (m, 4H), 3.0-3.2 (m, 4H), 4.12 (s, 2H), 5.45 (s, 2H), 6.6-6.7 (m, 2H), 6.88 (d, J = 8.1 Hz, 1H), 6.96 (d, J = 7.8 Hz, 1H), 7.1-7.2 (m, 2H), 7.2-7.3 (m, 2H), 7.4-7.6 (m, 4H), 7.6-7.8 (m, 3H), 8.53 (s, 1H).

Compound 48:

{2-Morpholinomethyl-8-(2-phenylbenzimidazol-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine monooxalate)

^{1}H NMR (DMSO-d_6) δ(ppm): 2.7-3.0 (m, 8H), 3.6-3.8 (m, 4H), 3.83 (s, 2H), 5.42 (s, 2H), 6.65-6.7 (m, 2H), 6.85 (d, J = 8.2 Hz, 1H), 6.92 (d, J = 8.1 Hz, 1H), 7.0-7.1 (m, 2H), 7.2-7.3

(m, 2H), 7.4-7.6 (m, 4H), 7.65-7.8 (m, 3H), 8.44 (s, 1H).

Compound 53:

{2-(2-Methylbenzimidazol-1-ylmethyl)-8-(1,2,5,6-tetrahydropyridin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

^{1}H NMR (DMSO-d_6) δ(ppm): 2.0-2.1 (m, 2H), 2.45 (t, J = 5.6 Hz, 2H), 2.54 (s, 3H), 2.75-2.85 (m, 2H), 2.85-3.0 (m, 4H), 3.35 (s, 2H), 5.28 (s, 2H), 5.55-5.75 (m, 2H), 6.8-7.0 (m, 6H), 7.1-7.2 (m, 2H), 7.4-7.6 (m, 2H), 8.28 (s, 1H).

Compound 54:

{2-(2-Methylbenzimidazol-1-ylmethyl)-8-(pyrrolidin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

^{1}H NMR (DMSO-d_6) δ(ppm): 1.5-1.8 (m, 4H), 2.3-2.5 (m, 4H), 2.54 (s, 3H), 2.8-3.0 (m, 4H), 3.39 (s, 2H), 5.28 (s, 2H), 6.7-6.9 (m, 6H), 7.1-7.2 (m, 2H), 7.3-7.5 (m, 2H), 8.25 (s, 1H).

Compound 60:

{2-(2-Methylbenzimidazo-1-ylmethyl)-8-morpholinomethyl-10,11-dihydro-5H-dibenz[b,f]azepine}

^{1}H NMR (DMSO-d_6) δ(ppm): 2.2-2.4 (m, 4H), 2.49 (s, 3H), 2.8-3.0 (m, 4H), 3.28 (s, 2H), 3.5-3.6 (m, 4H), 5.28 (s, 2H), 6.8-7.0 (m, 6H), 7.1-7.2 (m, 2H), 7.5-7.6 (m, 2H), 8.28 (s, 1H).

Compound 65:

{2-(5,6-Dimethylbenzimidazol-1-ylmethyl)-8-(1,2,5,6-tetrahydropyridin-1-ylmethyl)-10,11-dihydro-5H-dibenz

[b,f]azepine}

^{1}H NMR (DMSO-d_6) δ(ppm): 2.0-2.1 (m, 2H), 2.2-2.4 (m, 6H), 2.45 (t, J = 5.2 Hz, 2H), 2.75-2.85 (m, 2H), 2.85-3.05 (m, 4H), 3.30 (s, 2H), 5.24 (s, 2H), 5.6-5.7 (m, 2H), 6.8-7.0 (m, 6H), 7.31 (s, 1H), 7.40 (s, 1H), 8.17 (s, 1H), 8.27 (s, 1H).

Compound 66:

{2-(5,6-Dimethylbenzimidazol-1-ylmethyl)-8-(pyrrolidin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

^{1}H NMR (DMSO-d_6) δ(ppm): 1.5-1.8 (m, 4H), 2.27 (s, 3H), 2.28 (s, 3H), 2.3-2.4(m, 4H), 2.8-3.0 (m, 4H), 3.39 (s, 2H), 5.24 (s, 2H), 6.8-7.0 (m, 6H), 7.30 (s, 1H), 7.40 (s, 1H), 8.16 (s, 1H), 8.24 (s, 1H).

Compound 72:

{2-(5,6-Dimethylbenzimidazol-1-ylmethyl)-8-morpholino-methyl-10,11-dihydro-5H-dibenz[b,f]azepine}

^{1}H NMR (DMSO-d_6) δ(ppm): 2.2-2.4 (m, 10H), 2.8-3.0 (m, 4H), 3.28 (s, 2H), 3.5-3.6 (m, 4H), 5.24 (s, 2H), 6.8-7.0 (m, 6H), 7.30 (s, 1H), 7.39 (s, 1H), 8.16 (s, 1H), 8.28 (s, 1H).

Compound 77:

{2-(2-Ethylbenzimidazol-1-ylmethyl)-8-(1,2,5,6-tetrahydropyridin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

^{1}H NMR (DMSO-d_6) δ(ppm): 1.28 (t, J = 7.4 Hz, 3H), 1.95-2.05 (m, 2H), 2.43 (t, J = 5.4 Hz, 2H), 2.6-3.0 (m, 8H), 3.32 (s, 2H), 5.28 (s, 2H), 5.1-5.5 (m, 2H), 6.75-7.0 (m, 6H), 7.1-7.25

(m, 2H), 7.47 (m, 1H), 7.55 (m, 1H), 8.26 (s, 1H).

Compound 78:

{2-(2-Ethylbenzimidazol-1-ylmethyl)-8-(pyrrolidin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

^{1}H NMR (DMSO-d_6) δ(ppm): 1.28 (t, J = 7.4 Hz, 3H), 1.6-1.8 (m, 4H), 2.3-2.4 (m, 4H), 2.8-3.0 (m, 6H), 3.32 (s, 2H), 5.28 (s, 2H), 6.7-7.0 (m, 6H), 7.0-7.2 (m, 2H), 7.46 (m, 1H), 7.54 (m, 1H), 8.23 (s, 1H).

Compound 84:

{2-(2-Ethylbenzimidazo-1-ylmethyl)-8-morpholinomethyl-10,11-dihydro-5H-dibenz[b,f]azepine}

^{1}H NMR (DMSO-d_6) δ(ppm): 1.28 (t, J = 7.4 Hz, 3H), 2.2-2.4 (m, 4H), 2.8-3.0 (m, 6H), 3.27 (s, 2H), 3.5-3.6 (m, 4H), 5.27 (s, 2H), 6.7-7.0 (m, 6H), 7.1-7.2 (m, 2H), 7.47 (m, 1H), 7.55 (m, 1H), 8.26 (s, 1H).

Structures of compounds 88 to 90 are shown in Table 7 and analytical data thereof (APCI-MS and ^{1}H-NMR) are shown below.

Compound 88:

{2-(Imidazo[4,5-b]pyridin-1-ylmethyl)-8-(1,2,5,6-tetrahydropyridin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

APCI-MS: m/z 422 ([M + H]$^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 2.0-2.1 (m, 2H), 2.44 (t, J = 5.4 Hz, 2H), 2.75-2.8 (m, 2H), 2.8-3.0 (m, 4H), 3.30 (s, 2H), 5.33 (s, 2H), 5.5-5.6 (m, 2H), 6.8-7.0 (m, 4H), 7.0-7.05 (m,

2H), 7.27 (dd, J = 4.7 Hz, 8.0 Hz, 1H), 8.06 (d, J = 8.0 Hz, 1H), 8.27 (s, 1H), 8.37 (d, J = 4.7 Hz, 1H), 8.54 (s, 1H).

Compound 89:

{2-(Imidazo[4,5-b]pyridin-3-ylmethyl)-8-(1,2,5,6-tetrahydropyridin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine monooxalate}

APCI-MS: m/z 422 ($[M + H]^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 2.2-2.3 (m, 2H), 2.9-3.0 (m, 4H), 3.4-3.5 (m, 2H), 3.60 (t, J = 6.8 Hz, 2H), 4.05 (s, 2H), 5.37 (s, 2H), 5.67 (d, J = 10.8 Hz, 1H), 5.85 (d, J = 10.8 Hz, 1H), 6.9-7.0 (m, 2H), 7.0-7.1 (m, 4H), 7.25 (dd, J = 5.4, 8.1 Hz, 1H), 8.01 (d, J = 8.1 Hz, 1H), 8.40 (d, J = 5.4 Hz, 1H), 8.55 (s, 1H), 8.62 (s, 1H).

Compound 90:

{2-(Imidazo[4,5-c]pyridin-1-ylmethyl)-8-(1,2,5,6-tetrahydropyridin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

APCI-MS: m/z 422 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 2.1-2.2 (m, 2H), 2.56 (t, J = 5.7 Hz, 2H), 2.8-2.9 (m, 2H), 3.0-3.1 (m, 4H), 3.48 (s, 2H), 5.30 (s, 2H), 5.67 (d, J = 10.5 Hz, 1H), 5.73 (d, J = 10.5 Hz, 1H), 6.08 (s, 1H), 6.65-6.75 (m, 2H), 6.95-7.0 (m, 2H), 7.0-7.05 (m, 2H), 7.71 (d, J = 5.4 Hz, 1H), 8.02 (s, 1H), 8.45 (d, J = 5.4 Hz, 1H), 8.78 (s, 1H).

Referential Example 22: Synthesis of

N-methyl-N-(2-trityl-2H-tetrazol-5-ylmethyl)amine

2-Trityl-2H-tetrazol-5-ylmethanol (2.00 g, 5.84 mmol), N-methyl-2-nitrobenzenesulfonamide (1.64 g, 7.59 mmol) and triphenylphosphine (1.53 g, 5.84 mmol) were dissolved in a mixed solvent of tetrahydrofuran (30 mL) and toluene (20 mL), then a solution of diethyl azodicarboxylate in toluene (40%, 2.65 mL, 5.84 mmol) was added thereto and the mixture was stirred at room temperature for throughout a night. After the mixture was passed through a silica gel column (eluting solvent: ethyl acetate/hexane = 40/60) to remove original point components, acetone (5 mL) and acetonitrile (25 mL) were added to the residue prepared by concentrating *in vacuo*.

Mercaptoacetic acid (0.73 mL, 11 mmol) and 1,8-diazabicyclo[5,4,0]undec-7-ene (3.1 mL, 21 mmol) were added to the resulting suspension and the mixture was stirred at 60°C for 7 hours. The reaction solution was concentrated and the residue was dissolved in ethyl acetate. The resulting solution was washed with a saturated aqueous solution of sodium bicarbonate and a saturated saline solution successively, dried over anhydrous magnesium sulfate and concentrated. The residue was purified by a silica gel chromatography (eluting solvent: triethylamine/ethyl acetate = 1/99) to prepare N-methyl-N-(2-trityl-2H-tetrazol-5-yl)methylamine (396 mg, 1.11 mmol, yield: 19.0%).

^{1}H NMR ($CDCl_3$) δ(ppm): 2.45 (s, 3H), 4.07 (s, 2H), 7.07-7.36

(m, 15H).

Referential Example 23: Synthesis of compound 92 {[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl] acetate}

Compound 13 (7.98 g, 13.1 mmol) prepared in Referential Example 6 was dissolved in dimethyl sulfoxide (87 mL), lithium acetate (4.33 g, 65.7 mL) was added thereto and the mixture was stirred at 70°C for 2 days. The reaction solution was diluted with ethyl acetate, washed with water (for three times) and a saturated saline solution successively, dried over anhydrous magnesium sulfate and concentrated. The residue was purified using a silica gel chromatography (eluting solvent: ethyl acetate) to concentrate a fraction containing the objective product, ethanol was added to the residue and the resulting suspension was stirred at room temperature for 0.5 hour. The crystals separated out therefrom were filtered to give compound 92 (2.87 g, 6.31 mmol, yield: 48%).

APCI-MS: m/z 455 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 2.06 (s, 3H), 2.60 (s, 3H), 2.62 (s, 3H), 2.79 (q, J = 7.5 Hz, 2H), 2.98 (m, 4H), 4.98 (s, 2H), 5.34 (s, 2H), 6.13 (s, 1H), 6.58-6.83 (m, 4H), 6.88 (s, 1H), 7.01-7.07 (m, 2H).

Referential Example 24: Synthesis of compound 93 {[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-

ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-yl] methanol}

Compound 92 (2.79 g, 6.14 mmol) prepared in Referential Example 23 was suspended in tetrahydrofuran (61 mL), then a methanolic solution of sodium methoxide (28%, 6.2 mL, 31 mmol) was added and the mixture was stirred at room temperature for 3.5 hours. After confirming the progress of the reaction by a thin-layer chromatography, water was added to the reaction solution and the mixture was stirred at room temperature for 0.5 hour. The crystals precipitated therefrom were filtered, dried *in vacuo* and suspended in ethanol and the suspension was stirred for 1 hour under reflux and then stirred for another 1 hour at room temperature. The crystals separated out therefrom were filtered and dried *in vacuo* to give compound 93 (2.04 g, 4.95 mmol, yield: 81%).

APCI-MS: m/z 413 ($[M + H]^+$)

^1H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.6 Hz, 3H), 1.56 (t, J = 5.6 Hz, 1H), 2.60 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.6 Hz, 2H), 2.98 (m, 4H), 4.55 (d, J = 5.6 Hz, 2H), 5.34 (s, 2H), 6.03 (s, 1H), 6.59-6.85 (m, 3H), 6.88 (s, 1H), 7.03 (m, 2H).

Referential Example 25: Synthesis of compound 94 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-methoxymethyl-10,11-dihydro-5H-dibenz[b,f] azepine}

Methanol (20 μL, 0.50 mmol) was added to a suspension

of sodium hydride (55%, 11 mg, 0.25 mmol) in tetrahydrofuran (0.40 mL) and the mixture was stirred at room temperature for 20 minutes. After that, the reaction solution was added to a suspension of compound 13 (30 mg, 0.050 mmol) prepared in Referential Example 6 in tetrahydrofuran (0.20 mL) and the reaction was conducted at 60°C for 3.5 hours. After concentrating the reaction solution, the residue was dissolved in chloroform, the resulting solution was washed with water and a saturated saline solution successively, dried over anhydrous magnesium sulfate and concentrated. The residue was purified by a silica gel chromatography (eluting solvent: ethyl acetate/hexane/triethylamine = 45/50/5) to give compound 94 (6.5 mg, 15 mmol, yield: 30%).

APCI-MS: m/z 427 ($[M + H]^+$)

^1H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.6 Hz, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.6 Hz, 2H), 2.98 (m, 4H), 3.36 (s, 3H), 4.32 (s, 2H), 5.34 (s, 2H), 6.09 (s, 1H), 6.58-6.82 (m, 4H), 6.88 (s, 1H), 7.01 (m, 2H).

Referential Example 26: Synthesis of compound 95 {2-Allyloxymethyl-8-(2-ethyl-5,7-dimethyl-3H-imidazo [4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f] azepine}

The operation smilar to that in Referential Example 25 was conducted using allyl alcohol instead of methanol to give compound 95 in a yield of 34%.

APCI-MS: m/z 453 ([M + H]$^+$)

^{1}H NMR ($CDCl_3$)δ(ppm): 1.30 (t, J = 7.6 Hz, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.80 (q, J = 7.6 Hz, 2H), 2.98 (m, 4H), 4.00 (dt, J = 5.6, 1.5 Hz, 2H), 4.39 (s, 2H), 5.19 (dq, J = 10.2, 1.5 Hz, 1H), 5.29 (dq, J = 17.0, 1.5 Hz, 1H), 5.34 (s, 2H), 5.95 (m, 1H), 6.10 (s, 1H), 6.58-6.83 (m, 4H), 6.88 (s, 1H), 7.03 (m, 2H).

Referential Example 27: Synthesis of compound 96 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-(2-methoxyethoxymethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

The operation similar to that in Referential Example 25 was conducted using 2-methoxyethanol instead of methanol to give compound 96 in a yield of 9.3%.

APCI-MS: m/z 495 ([M + H]$^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.80 (q, J = 7.5 Hz, 2H), 2.98 (m, 4H), 3.38 (s, 3H), 3.57 (m, 4H), 4.44 (s, 2H), 5.34 (s, 2H), 6.01 (s, 1H), 6.62 (d, J = 8.6 Hz, 1H), 6.67 (d, J = 8.1 Hz, 1H), 6.82 (m, 2H), 6.88 (s, 1H), 7.00-7.06 (m, 2H).

Referential Example 28: Synthesis of compound 97 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-(2,2,2-trifluoroethoxymethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

The operation similar to that in Referential Example 25

was conducted using 2,2,2-trifluoroethanol instead of methanol to give compound 97 in a yield of 64%.

APCI-MS: m/z 495 ($[M + H]^+$)

^1H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.6 Hz, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.6 Hz, 2H), 2.98 (m, 4H), 3.78 (q, J = 8.7 Hz, 2H), 4.54 (s, 2H), 5.34 (s, 2H), 6.24 (s, 1H), 6.60 (d, J = 7.8 Hz, 1H), 6.71 (d, J = 8.1 Hz, 1H), 6.76-6.82 (m, 2H), 6.89 (s, 1H), 6.98-7.04 (m, 2H)

Referential Example 29: Synthesis of compound 98 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-(2-methylpropoxymethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

The operation similar to that in Referential Example 25 was conducted using 2-methyl-1-propanol instead of methanol to give compound 98 in a yield of 11%.

APCI-MS: m/z 469 ($[M + H]^+$)

^1H NMR ($CDCl_3$) δ(ppm): 0.91 (d, J = 6.7 Hz, 6H), 1.30 (t, J = 7.4 Hz, 3H), 1.89 (m, 1H), 2.60 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.4 Hz, 2H), 2.99 (m, 4H), 3.20 (d, J = 6.5 Hz, 2H), 4.37 (s, 2H), 5.34 (s, 2H), 6.01 (s, 1H), 6.60 (d, J = 8.9 Hz, 1H), 6.67 (d, J = 7.8 Hz, 1H), 6.81 (m, 2H), 6.88 (s, 1H), 6.98-7.05 (m, 2H).

Referential Example 30: Synthesis of compound 99 {2-Benzyloxymethyl-8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]

azepine}

The operation similar to that in Referential Example 25 was conducted using benzyl alcohol instead of methanol to give compound 99 in a yield of 78%.

APCI-MS: m/z 503 ([M + H]$^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 2.60 (s, 3H), 2.62 (s, 2H), 2.79 (q, J = 7.5 Hz, 2H), 2.97 (m, 4H), 4.42 (s, 2H), 4.53 (s, 2H), 5.33 (s, 2H), 6.20 (s, 1H), 6.59 (d, J = 7.9 Hz, 1H), 6.69 (d, J = 7.9 Hz, 1H), 6.78 (m, 2H), 6.88 (s, 1H), 7.02 (m, 2H), 7.26-7.36 (m, 5H).

Referential Example 31: Synthesis of compound 100 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-(2-phenylethoxymethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

The operation similar to that in Referential Example 25 was conducted using 2-phenylethanol instead of methanol to give compound 100 in a yield of 38%.

APCI-MS: m/z 517 ([M + H]$^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.80 (q, J = 7.5 Hz, 2H), 2.91 (t, J = 7.2 Hz, 2H), 2.97 (m, 4H), 3.66 (t, J = 7.2 Hz, 2H), 4.39 (s, 2H), 5.34 (s, 2H), 6.08 (s, 1H), 6.60 (d, J = 8.7 Hz, 1H), 6.66 (d, J = 8.1 Hz, 1H), 6.80 (m, 2H), 6.88 (s, 1H), 6.94-7.01 (m, 2H), 7.19-7.30 (m, 5H).

Referential Example 32: Synthesis of compound 101

{2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-(pyridin-2-ylmethoxymethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

The operation similar to that in Referential Example 25 was conducted using pyridin-2-ylmethanol instead of methanol to give compound 101 in a yield of 65%.

APCI-MS: m/z 504 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.80 (q, J = 7.5 Hz, 2H), 2.98 (m, 4H), 4.52 (s, 2H), 4.66 (s, 2H), 5.34 (s, 2H), 6.25 (s, 1H), 6.60 (d, J = 7.9 Hz, 1H), 6.70 (d, J = 7.9 Hz, 1H), 6.76-6.81 (m, 2H), 6.88 (s, 1H), 7.03-7.08 (m, 2H), 7.18 (br dd, J = 7.6, 4.8 Hz, 1H), 7.47 (d, J = 7.9 Hz, 1H), 7.68 (td, J = 7.7, 1.8 Hz, 1H), 8.54 (br d, J = 4.8 Hz, 1H).

Referential Example 33: Synthesis of compound 102 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-(furan-2-ylmethoxymethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

The operation similar to that in Referential Example 25 was conducted using furan-2-ylmethanol instead of methanol to give compound 102 in a yield of 77%.

APCI-MS: m/z 493 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 2.60 (s, 3H), 2.62 (s, 3H), 2.79 (q, J = 7.5 Hz, 2H), 2.97 (m, 4H), 4.41 (s, 2H), 4.45 (s, 2H), 5.33 (s, 2H), 6.21 (br s, 1H), 6.31 (dd,

J = 3.1, 0.8 Hz, 1H), 6.33 (dd, J = 3.1, 1.8 Hz, 1H), 6.58 (d, J = 8.1 Hz, 1H), 6.69 (d, J = 7.9 Hz, 1H), 6.75-6.80 (m, 2H), 6.88 (s, 1H), 7.00-7.04 (m, 2H), 7.40 (dd, J = 1.8, 0.8 Hz, 1H).

Referential Example 34: Synthesis of compound 103 {8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine-2-carbonitrile}

8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-carboaldehyde (650 mg, 1.58 mmol) prepared in step 1 of Referential Example 15 was suspended in acetonitrile (16 mL), then hydroxylamine hydrochloride (153 mg, 2.38 mmol), triethylamine (0.331 mL, 2.38 mmol) and phthalic anhydride (328 mg, 2.21 mmol) were added thereto and the mixture was stirred at 80°C for one night. The reaction solution was concentrated, the residue was dissolved in chloroform, the resulting solution was washed with an aqueous ammonia solution (3%) and a saturated saline solution successively, dried over anhydrous magnesium sulfate and concentrated. The residue was purified by a silica gel chromatography (eluting solvent: methanol/chloroform = 1/99), the fraction containing the objective product was concentrated, ethanol was added to the residue and the resulting suspension was stirred at 60°C for 0.5 hour and stirred at room temperature for 1 hour. The crystals precipitated therefrom

were filtered and dried *in vacuo* to give compound 103 (440 mg, 1.08 mmol, yield: 68%).

APCI-MS: m/z 408 ([M + H]$^+$)

^{1}H NMR ($CDCl_3$)δ (ppm): 1.31 (t, J = 7.6 Hz, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.6 Hz, 2H), 2.98 (m, 4H), 5.36 (s, 2H), 6.48 (s, 1H), 6.63-6.90 (m, 5H), 7.28-7.33 (m, 2H).

Referential Example 35: Synthesis of compound 104 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-(2H-tetrazol-5-yl)-10,11-dihydro-5H-dibenz [b,f]azepine}

Compound 103 prepared in Referential Example 34 was used and, by the similar manner as in the latter part of Referential Example 20, compound 104 was obtained in a yield of 72%

APCI-MS: m/z 451 ([M + H]$^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.24 (t, J = 7.4 Hz, 3H), 2.48-2.53 (s x 2, 6H, overlapped with DMSO), 2.80 (q, J = 7.4 Hz, 2H), 2.86-3.02 (m, 4H), 5.32 (s, 2H), 6.83 (dd, J = 8.1, 2.1 Hz, 1H), 6.91-6.98 (m, 3H), 7.10 (d, J = 9.0 Hz, 1H), 7.65-7.70 (m, 2H), 8.20 (s, 1H).

Referential Example 36: Synthesis of compound 105 {[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-yl] acetonitrile}

Compound 13 (2.04 g, 3.36 mmol) prepared in Referential Example 6 was dissolved in dimethylformamide (17 mL), sodium

cyanide (361 mg, 7.37 mmol) was added thereto and the mixture was stirred at 50°C for 10 hours. The reaction solution was cooled to room temperature, diluted with ethyl acetate, washed with a 2 mol/L aqueous solution of sodium hydroxide, water (two times) and a saturated saline solution successively, dried over anhydrous magnesium sulfate and concentrated. The residue was recrystallized from ethanol to give compound 105 (751 mg, 1.78 mmol, yield: 53%).

APCI-MS: m/z 422 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.31 (t, J = 7. 5Hz, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.5 Hz, 2H), 2.99 (m, 4H), 3.62 (s, 2H), 5.34 (s, 2H), 6.01 (s, 1H), 6.59-6.71 (m, 2H), 6.80-6.84 (m, 2H), 6.88 (s, 1H), 6.95-7.01 (m, 2H).

Referential Example 37: Synthesis of compound 106 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-8-(2H-tetrazol-5-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

Compound 105 prepared in Referential Example 36 was used and, by the similar manner as in the latter part of Referential Example 20, compound 106 was obtained in a yield of 76%

APCI-MS: m/z 465 ($[M + H]^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.22 (t, J = 7.6 Hz, 3H), 2.48-2.52 (s x 2, 6H, overlapped with DMSO), 2.78 (q, J = 7.6 Hz, 2H), 2.86 (m, 4H), 4.11 (s, 2H), 5.28 (s, 2H), 6.75-6.94 (m, 7H), 8.32 (br s, 1H).

Referential Example 38: Synthesis of compound 107 {[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-yl]acetic acid}

Compound 105 (247 mg, 0.586 mmol) prepared in Referential Example 36 was suspended in ethanol (12 mL), sodium hydroxide (938 mg, 23.5 mmol) was added thereto and the mixture was stirred for 3 hours under a condition of heating to reflux. After confirming the progress of the reaction by a thin-layer chromatography, the reaction solution was cooled to room temperature and pH was adjusted to 5 with 1 mol/L hydrochloric acid. The crystals separated out therefrom were filtered, dried *in vacuo*, suspended in ethanol, stirred at 60°C for 0.5 hour and stirred at room temperature for 1 hour. The crystals separated out were filtered and dried *in vacuo* to give compound 107 (122 mg, 0.243 mmol, yield: 41%).

APCI-MS: m/z 441 ($[M + H]^+$)

^1H NMR (DMSO-d_6) δ(ppm): 1.23 (t, J = 7.5 Hz, 3H), 2.48-2.53 (s x 2, 6H, overlapped with DMSO), 2.78 (q, J = 7.5 Hz, 2H), 2.87 (br s, 4H), 3.38 (s, 2H), 5.38 (s, 2H), 6.74-6.94 (m, 7H), 8.27 (s, 1H), 12.15 (br s, 1H).

Referential Example 39: Synthesis of compound 108 {Methyl [8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl-sulfanyl]acetate}

Compound 13 (1.04 g, 1.71 mmol) prepared in Referential Example 6 was dissolved in chloroform (17 mL), then methyl mercaptoacetate (0.199 mL, 2.23 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.384 mL, 2.57 mmol) were added thereto and the mixture was stirred at 40°C for 7 hours. The reaction solution was concentrated, the residue was purified by a silica gel chromatography (eluting solvent: methanol/chloroform = 1/99) and a fraction containing compound was concentrated. Ethanol was added to the residue and the resulting suspension was stirred at 60°C for 0.5 hour and then at room temperature for 1 hour. The crystals separated out were filtered to give compound 108 (628 mg, 1.25 mmol, yield: 73%).

APCI-MS: m/z 501 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$)δ(ppm): 1.30 (t, J = 7.5 Hz, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.80 (q, J = 7.5 Hz, 2H), 2.98 (m, 4H), 3.09 (s, 2H), 3.72 (s, 3H), 3.73 (s, 2H), 5.34 (s, 2H), 6.01 (s, 1H), 6.59-6.67 (m, 2H), 6.82 (m, 2H), 6.88 (s, 1H), 6.96-7.03 (m, 2H)

Referential Example 40: Synthesis of compound 109 {[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethylsulfanyl]acetic acid}

Compound 108 (350 mg, 0.699 mmol) prepared in Referential Example 39 was used and, by the similar manner as in Referential

Example 12, compound 109 was obtained in a yield of 38%

APCI-MS: m/z 487 ([M + H]$^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.16 (t, J = 7.4 Hz, 3H), 2.42-2.50 (s x 2, 6H, overlapped with DMSO), 2.81 (q, J = 7.4 Hz, 2H), 2.88 (m, 6H), 3.49 (s, 2H), 5.22 (s, 2H), 6.67-6.89 (m, 7H), 8.18 (s, 1H).

Referential Example 41: Synthesis of compound 110 {Ethyl 8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-yl-methyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-carboxylate}

Step 1:

1-(10,11-Dihydro-5H-dibenz[b,f]azepin-2-ylmethyl)-1-methylpiperidinium iodide (6.68 g, 15.4 mmol) was dissolved in dimethyl sulfoxide (110 mL), lithium acetate (5.07 g, 76.9 mmol) was added thereto and the mixture was stirred at 70°C for 2 days. The reaction solution was diluted with ethyl acetate, washed with water (for three times) and a saturated saline solution successively, dried over anhydrous magnesium sulfate and concentrated. The residue was purified by a silica gel chromatography (eluting solvent: ethyl acetate/hexane = 30/70) to give (10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl) acetate (2.85 g, 10.7 mmol, yielde: 69%).

APCI-MS: m/z 268 ([M + H]$^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 2.07 (s, 3H), 3.07 (br s, 4H), 4.99 (s, 2H), 6.05 (br s, 1H), 6.66-6.85 (m, 3H), 7.02-7.11 (m, 4H).

Step 2:

(10,11-Dihydro-5H-dibenz[b,f]azepin-2-ylmethyl) acetate (2.85 g, 10.7 mmol) prepared in the step 1 was suspended in methanol (110 mL), then a methanolic solution of sodium methoxide (38%, 1.14 mL, 5.36 mmol) was added thereto and the mixture was stirred at room temperature for 1 hour. The reaction solution was concentrated, a saturated saline solution and chloroform were added to the residue and extraction was conducted with chloroform for three times. The organic layers were combined, dried over anhydrous magnesium sulfate and concentrated. The residue was recrystallized from diisopropyl ether to give 10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethanol (1.73 g, 7.68 mmol, yield: 72%).

APCI-MS: m/z 226 ($[M + H]^+$)

^1H NMR ($CDCl_3$)δ(ppm): 1.49 (t, J = 5.8 Hz, 1H), 3.08 (br s, 4H), 4.57 (d, J = 5.8 Hz, 2H), 6.02 (br s, 1H), 6.66-6.87 (m, 3H), 7.02-7.11 (m, 4H).

Step 3:

10,11-Dihydro-5H-dibenz[b,f]azepin-2-ylmethanol (6.1 g, 70 mmol) prepared in the step 2 was dissolved in chloroform (77 mL), then manganese dioxide (4.55 g, 46.1 mmol) was added thereto and the mixture was stirred at room temperature for 8 hours. The reaction solution was filtered through Celite and the filtrate was concentrated. The residue was purifed by a silica gel chromatography (eluting solvent: ethyl

acetate/hexane = 20/80) to give 10,11-dihydro-5H-dibenz[b,f]azepin-2-carboaldehyde (1.15 g, 5.15 mmol, yield: 67%).

APCI-MS: m/z 224 ($[M + H]^+$)

^1H NMR ($CDCl_3$) δ(ppm): 3.11 (m, 4H), 6.49 (br s, 1H), 6.87-6.91 (m, 3H), 7.07-7.17 (m, 2H), 7.55-7.62 (m, 2H), 9.88 (s, 1H).

Step 4:

10,11-Dihydro-5H-dibenz[b,f]azepin-2-carboaldehyde (665 mg, 2.98 mmol) prepared in the step 3 was dissolved in a mixed solvent of acetonitrile (18 mL) and water (18 mL), then dimethyl sulfoxide (2.1 mL, 30 mmol), sodium dihydrogen phosphate (1.43 g, 11.9 mmol) and sodium chlorite (404 mg, 4.47 mmol) were added thereto and the mixture was stirred at 50°C for 4 hours. Ethyl acetate and water were added to the reaction solution and extraction was conducted with ethyl acetate for two times. The organic layers were combined, washed with water, dried over anhydrous magnesium sulfate and concentrated. A mixed solvent (3:1) of ethyl acetate and hexane was added to the residue and the resulting suspension was stirred at 60°C for 0.5 hour and stirred at room temperature for 1 hour. The crystals separated out were filtered to give 10,11-dihydro-5H-dibenz[b,f]azepin-2-carboxylic acid (598 mg, 2.50 mmol, yield: 84%).

APCI-MS: m/z 240 ($[M + H]^+$)

^1H NMR ($CDCl_3$) δ(ppm): 3.11 (m, 4H), 6.39 (br s, 1H), 6.77-6.80 (m, 3H), 7.06-7.16 (m, 2H), 7.79-7.84 (m, 2H).

Step 5:

10,11-Dihydro-5H-dibenz[b,f]azepin-2-carboxylic acid (426 mg, 1.78 mmol) prepared in the step 4 was dissolved in ethanol (8.9 mL), thionyl chloride (0.26 mL, 3.6 mmol) was added thereto and the mixture was stirred for 5 hours with heating to reflux. The reaction solution was concentrated, chloroform and a saturated aqueous solution of sodium bicarbonate were added thereto and extraction with chloroform was conducted for three times. The organic layers were combined, washed with a saturated saline solution, dried over anhydrous magnesium sulfate and concentrated. The residue was purified by a silica gel chromatography (eluting solvent: ethyl acetate/hexane = 10/90) to give ethyl 10,11-dihydro-5H-dibenz[b,f]azepin-2-carboxylate (383 mg, 1.43 mmol, yield: 81%).

APCI-MS: m/z 268 ($[M + H]^+$)

^1H NMR ($CDCl_3$) δ(ppm): 1.37 (t, J = 7.0 Hz, 3H), 3.09 (m, 4H), 4.33 (q, J = 7.0 Hz, 2H), 6.34 (br s, 1H), 6.69-6.86 (m, 3H), 7.04-7.14 (m, 2H), 6.72-6.78 (m, 2H).

Step 6:

Ethyl 10,11-dihydro-5H-dibenz[b,f]azepin-2-carboxylate (443 mg, 1.66 mmol) prepared in the step 5 was dissolved in a mixed solvent

of chloroform (8.3 mL) and acetic acid (8.3 mL), then piperidine (0.573 mL, 5.80 mmol) and paraformaldehyde (149 mg, 4.97 mmol) were added thereto and the mixture was heated at 60°C and stirred for 1.5 days. The reaction solution was concentrated, ethyl acetate and a saturated aqueous saline solution were added thereto and extraction with ethyl acetate was conducted. The organic layers were combined, washed with a saturated saline solution, dried over anhydrous sodium sulfate and concentrated. The residue was purified by a silica gel chromatography (eluting solvent: ethyl acetate/hexane/triethylamine = 70/25/5) and a fraction containing the objective substance was concentrated. The residue was subjected to trituration with diethyl ether to give ethyl 8-piperidinomethyl-10,11-dihydro-5H-dibenz[b,f]azepin-2-carboxylate (249 mg, 0.683 mmol, yield: 41%).

APCI-MS: m/z 365 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.34-1.46 (m, 5H), 1.67 (m, 4H), 2.36 (br s, 4H), 3.08 (m, 4H), 3.38 (s, 2H), 4.33 (q, J = 7.1 Hz, 2H), 6.32 (s, 1H), 6.67-6.74 (m, 2H), 6.99-7.06 (m, 2H), 7.72-7.76 (m, 2H).

Step 7

Ethyl 8-piperidinomethyl-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxylate (231 mg, 0.634 mmol) prepared in the step 6 was dissolved in dichloromethane (3.2 mL), methyl iodide (59.2 μL,

0.951 mmol) was added thereto and the mixture was stirred at room temperature for one night. The reaction solution was concentrated *in vacuo* to give 1-(8-ethoxycarbonyl-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl)-1-methylpiperidinium iodide (321 mg, 0.634 mmol, yield: 100%).

^{1}H NMR ($CDCl_3$) δ(ppm): 1.37 (t, J = 7.1 Hz, 3H), 1.75-1.95 (m, 6H), 2.96 (br s, 4H), 3.11 (s, 3H), 3.50 (m, 2H), 3.70 (m, 2H), 4.32 (q, J = 7.1 Hz, 2H), 4.90 (s, 2H), 7.14-7.35 (m, 4H), 7.49 (s, 1H), 7.71 (m, 2H).

Step 8:

2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridine (180 mg, 1.03 mmol) was dissolved in dimethylformamide (0.60 mL), sodium hydride (55%, 33.6 mg, 0.770 mmol) was added thereto with stirring by dividing into several times and the mixture was stirred at 50°C for 0.5 hour. The reaction solution was cooled down to room temperature, then a solution of 1-(8-ethoxycarbonyl-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl)-1-methylpiperidinium iodide (130 mg, 0.256 mmol) prepared in the step 7 dissolved in dimethylformamide (1.2 mL) was added thereto and the mixture was stirred at room temperature for 1 hour. The reaction solution was diluted with ethyl acetate, washed with water, water and a saturated aqueous saline solution successively, dried over anhydrous magnesium sulfate and concentrated. The residue was purified by a silica gel

chromatography (eluting solvent: methanol/chloroform = 1/99) and a fraction containing the objective substance was concentrated. Diethyl ether was added to the residue and the mixture was stirred for 0.5 hours with heating to reflux and, after that, stirred at room temperature for 1 hour. The crystals separated out therefrom were filtered to give compound 110 (76.7 mg, 0.169 mmol, yield: 66%).

APCI-MS: m/z 455 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.31 (t, J = 7.6 Hz, 3H), 1.36 (t, J = 7.1 Hz, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.80 (q, J = 7.6 Hz, 2H), 2.97 (m, 2H), 3.40 (m, 2H), 4.32 (q, J = 7.1 Hz, 2H), 5.36 (s, 2H), 6.35 (s, 1H), 6.64-6.71 (m, 2H), 6.82-6.90 (m, 3H), 7.70-7.74 (m, 2H).

Referential Example 42: Synthesis of compound 111 {8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxylic acid}

The operation similar to that in Referential Example 12 was conducted using compound 110 (900 mg, 1.98 mmol) prepared in Referential Example 41 to give compound 111 in a yield of 97%.

APCI-MS: m/z 427 ($[M + H]^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.24 (t, J = 7.5 Hz, 3H), 2.51-2.54 (s x 2, 6H, overlapped with DMSO), 2.82-2.99 (m, 6H), 5.37 (s, 2H), 6.84-7.03 (m, 5H), 7.58 (m, 2H), 8.87 (br s, 1H), 12.25

(br s, 1H).

Referential Example 43: Synthesis of compound 112 {[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-yl](4-methylpiperazin-1-yl)methanone}

Compound 111 (100 mg, 0.234 mmol) prepared in Referential Example 42 was dissolved in a mixed solvent of dimethylformamide (2.3 mL) and tetrahydrofuran (4.6 mL), then 4-methylpiperazine (39 μL, 0.352 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide monohydrochloride (89.7 mg, 0.468 mmol) and 1-hydroxybenzotriazole (35.8 mg, 0.234 mmol) were added thereto and the mixture was stirred at room temperature for 8 hours. After confirming the progress of the reaction by a thin-layer chromatography, the reaction solution was concentrated. The residue was dissolved in chloroform and the resulting solution was washed with water (for two times), a saturated sodium bicarbonate solution and a saturated saline solution successively, dried over anhydrous magnesium sulfate and concentrated. Diethyl ether was added to the residue, the resulting suspension was stirred at room temperature for 1 hour and solid was filtered to give compound 112 (47.7 mg, 0.0938 mmol, yield: 40%).

APCI-MS: m/z 509 ($[M + H]^+$)

^1H NMR ($CDCl_3$)δ(ppm): 1.31 (t, J = 7.5 Hz, 3H), 2.33 (s,

3H), 2.43 (br s, 4H), 2.60 (s, 3H), 2.63 (s, 3H), 2.80 (q, J = 7.5 Hz, 2H), 2.99 (m, 4H), 3.66 (br s, 4H), 5.35 (s, 2H), 6.18 (s, 1H), 6.62-6.69 (m, 2H), 6.83 (m, 2H), 6.85 (s, 1H), 7.10-7.15 (m, 2H).

Referential Example 44: Synthesis of compound 113 {[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-yl] (pyrrolidine-1-yl)methanone}

The operation similar to that in Referential Example 43 was conducted using pyrrolidine instead of 4-methylpiperazine to give compound 113 in a yield of 90%.

APCI-MS: m/z 480 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.31 (t, J = 7.5 Hz, 3H), 1.88 (br s, 4H), 2.60 (s, 3H), 2.63 (s, 3H), 2.80 (q, J = 7.5 Hz, 2H), 2.99 (m, 4H), 3.56 (m, 4H), 5.35 (s, 2H), 6.19 (s, 1H), 6.62-6.69 (m, 2H), 6.81-6.86 (m, 2H), 6.89 (s, 1H), 7.24-7.29 (m, 2H).

Referential Example 45: Synthesis of compound 114 {[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-yl] (4-hydroxypiperidino)methanone}

The operation similar to that in Referential Example 43 was conducted using 4-piperidinol instead of 4-methylpiperazine to give compound 114 in a yield of 62%.

APCI-MS: m/z 510 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$)δ(ppm): 1.31 (t, J = 7.0 Hz, 3H), 1.48-1.58

(m, 2H), 1.86-1.97 (m, 2H), 2.60 (s, 3H), 2.63 (s, 2H), 2.80 (q, J = 7.0 Hz, 2H), 2.99 (m, 4H), 3.22-3.33 (m, 2H), 3.91-4.00 (m, 3H), 5.36 (s, 2H), 6.21 (s, 1H), 6.62-6.70 (m, 2H), 6.81-6.85 (m, 2H), 6.89 (s, 1H), 7.08-7.14 (m, 2H).

Referential Example 46: Synthesis of compound 115 {[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-carboxylic acid (2-hydroxyethyl)amide}

The operation similar to that in Referential Example 43 was conducted using ethanolamine instead of 4-methylpiperazine to give compound 115 in a yield of 82%.

APCI-MS: m/z 470 ($[M + H]^+$)

^1H NMR ($CDCl_3$) δ(ppm): 1.31 (t, J = 7.5 Hz, 3H), 1.71 (br s, 1H), 2.60 (s, 3H), 2.63 (s, 3H), 2.79 (q, J = 7.5 Hz, 2H), 2.97 (m, 4H), 3.59 (m, 2H), 3.81 (t, J = 9.6 Hz, 2H), 5.35 (s, 2H), 6.41 (s, 1H), 6.54 (t, J = 5.6 Hz, 1H), 6.63-6.71 (m, 2H), 6.80-6.84 (m, 2H), 6.99 (s, 1H), 7.44-7.48 (m, 2H).

Referential Example 47: Synthesis of compound 116 {[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxylic acid[2-(pyrolidin-1-yl)ethyl]amide}

The operation similar to that in Referential Example 43 was conducted using 2-(pyrrolidine-1-yl)ethylamine instead of 4-methylpiperazine to give compound 116 in a yield of 92%.

APCI-MS: m/z 523 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.31 (t, J = 7.6 Hz, 3H), 1.78 (m, 4H), 1.57 (m, 4H), 2.60 (s, 3H), 2.63 (s, 3H), 2.70 (t, J = 5.9 Hz, 2H), 2.79 (q, J = 7.6 Hz, 2H), 2.97 (m, 2H), 3.04 (m, 2H), 3.53 (q, J = 5.7 Hz, 2H), 5.35 (s, 2H), 6.30 (s, 1H), 6.63-6.72 (m, 3H), 6.83 (m, 2H), 6.89 (s, 1H), 7.45-7.52 (m, 2H).

Referential Example 48: Synthesis of compound 117 {[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-yl](morpholino)methanone}

The operation similar to that in Referential Example 43 was conducted using morpholine instead of 4-methylpiperazine to give compound 117 in a yield of 98%.

APCI-MS: m/z 496 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.31 (t, J = 7.5 Hz, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.80 (q J = 7.5 Hz, 2H), 2.99 (m, 4H), 3.66 (m, 8H), 5.35 (s, 2H), 6.22 (s, 1H), 6.62-6.71 (m, 2H), 6.81-6.86 (m, 2H), 6.89 (s, 1H), 7.10-7.16 (m, 2H).

Referential Example 49: Synthesis of compound 118 {8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxylic acid bis(2-hydroxyethyl)amide}

The operation similar to that in Referential Example 43 was conducted using 2-(2-hydroxyethylamino)ethanol instead of 4-methylpiperazine to give compound 118 in a yield of 38%.

APCI-MS: m/z 514 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.21 (t, J = 7.5 Hz, 3H), 2.60 (s, 3H), 2.63 (s, 3H), 2.80 (q, J = 7.5 Hz, 2H), 2.98 (m, 4H), 3.23 (br s, 2H), 3.63 (br s, 4H), 3.87 (br s, 4H), 5.35 (s, 2H), 6.20 (s, 1H), 6.62-6.69 (m, 2H), 6.83 (m, 2H), 6.89 (s, 1H), 7.24-7.29 (m, 2H).

Referential Example 50: Synthesis of compound 119 {8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxylic acid amide}

The operation similar to that in Referential Example 43 was conducted using ammonia instead of 4-methylpiperazine to give compound 119 in a yield of 57%.

APCI-MS: m/z 426 ([M + H]$^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.23 (t, J = 7.4 Hz, 3H), 2.48-2.52 (s x 2, 6H, overlapped with DMSO), 2.78 (q, J = 7.4 Hz, 2H), 2.92 (br q, J = 7.3 Hz, 4H), 5.31 (s, 2H), 6.78-7.00 (m, 6H), 7.52-7.65 (m, 3H), 8.68 (s, 1H).

Referential Example 51: Synthesis of compound 120 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-8-(pyrrolidin-1-ylmethyl)-10,11-dihydro-5H-dibenz[b,f]azepine}

Compound 3 prepared in Referential Example 3 (400 mg, 0.876 mmol) was dissolved in acetic acid (8.8 mL), then paraformaldehyde (0.47 g, 16 mmol) and sodium cyanoborohydride (2.2 g, 10 mmol) were added thereto and the mixture was stirred

at room temperature for 5 hours. Chloroform and a saturated sodium bicarbonate solution were added to the reaction solution and the aqueous layer was extracted with chloroform twice. The organic layer was washed with a saturated saline solution, dried over anhydrous magnesium sulfate and concentrated. The residue was purified by an NH-silica gel chromatography (eluting solvent: chloroform/hexane = 50/50) to give compound 120 (342 mg, 0.713 mmol, yield: 81%).

APCI-MS: m/z 480 ($[M + H]^+$)

^1H NMR ($CDCl_3$) δ(ppm): 1.31 (t, J = 7.5 Hz, 3H), 1.76 (m, 4H), 2.47 (m, 4H), 2.58 (s, 3H), 2.62 (s, 3H), 2.78 (q, J = 7.5 Hz, 2H), 3.06 (m, 4H), 3.29 (s, 3H), 3.50 (s, 2H), 5.35 (s, 2H), 6.83-6.98 (m, 5H), 7.02-7.08 (m, 2H).

Referential Example 52: Synthesis of compound 121 {1-[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]piperidine-4-carboxylic acid}

Step 1:

Compound 16 (1.20 g, 2.18 mmol) prepared in Referential Example 9 was dissolved in acetic acid (10 mL), then paraformaldehyde (0.73 g, 21.8 mmol) and sodium cyanoborohydride (0.58 g, 8.70 mmol) were added thereto and the mixture was stirred at room temperature for 15 hours. Ethyl acetate and a 1 mol/L aqueous solution of sodium hydroxide were added to the reaction solution and the aqueous layer was

extracted with ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate and the solvent was evaporated *in vacuo*. The residue was purified by an NH-silica gel chromatography (eluting solvent: a mixed solvent of hexane-ethyl acetate) to give ethyl 1-[8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]piperidine-4-carboxylate (1.25 g, 2.18 mmol, yield: 100%).

APCI-MS: m/z 566 ($[M + H]^+$)

^{1}H NMR ($CDCl_3$) δ(ppm): 1.23 (t, J = 7.4 Hz, 3H), 1.31 (t, J = 7.6 Hz, 3H), 1.6-2.0 (m, 6H), 2.23 (m, 1H), 2.58 (s, 3H), 2.62 (s, 3H), 2.73 (q, J = 7.4 Hz, 2H), 2.75-2.9 (m, 2H), 3.0-3.15 (m, 4H), 3.28 (s, 3H), 3.36 (s, 2H), 4.10 (q, J = 7.6 Hz, 2H), 5.34 (s, 2H), 6.8-7.1 (m, 7H).

Step 2:

The operation similar to that in Referential Example 12 was conducted using ethyl 1-[8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]piperidine-4-carboxylate prepared in the step 1 to give compound 121 in a yield of 42%.

APCI-MS: m/z 538 ($[M + H]^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.23 (t, J = 7.4 Hz, 3H), 1.5-1.8 (m, 2H), 1.8-2.0 (m, 2H), 2.2-2.4 (m, 2H), 2.49 (s, 3H), 2.50

(s, 3H), 2.78 (q, J = 7.4 Hz, 2H), 2.8-3.05 (m, 8H), 3.22 (s, 2H), 3.5-3.9 (m, 2H), 5.34 (s, 2H), 6.85 (dd, J = 2.0, 8.4 Hz, 1H), 6.93 (s, 1H), 6.94 (d, J = 2.0 Hz, 1H), 7.0-7.2 (m, 4H).

Referential Example 53: Synthesis of compound 122 {2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-8-[4-(2H-tetrazol-5-yl) piperidinomethyl]-10,11-dihydro-5H-dibenz[b,f]azepine monohydrochloride}

The operation similar to that in the step 1 of Referential Example 52 was conducted using 1-[8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-yl methyl)-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]-piperidine-4-carbonitrile prepared in Referential Example 20 to prepare 1-[8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-10,11-dihydro-5H-dibenz[b,f]-azepin-2-ylmethyl]piperidine-4-carbonitrile in a yield of 92%. The operation similar to that in the latter part of Referential Example 20 was conducted using the above compound to prepare 2-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-8-[4-(2H-tetrazol-5-yl)piperidino-methyl]-10,11-dihydro-5H-dibenz[b,f]azepine in a yield of 10%. This was dissolved in chloroform, a solution of 4 mol/L of hydrogen chloride in ethyl acetate was added thereto and the solid separated out therefrom were filtered to prepare compound

122.

APCI-MS: m/z 562 ($[M + H]^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.28 (t, J = 7.6 Hz, 3H), 2.0-2.5 (m, 4H), 2.58 (s, 3H), 2.63 (s, 3H), 2.9-3.2 (m, 8H), 3.2-3.3 (m, 4H), 3.4-3.6 (m, 2H), 4.17 (s, 2H), 5.56 (s, 2H), 7.0-7.2 (m, 4H), 7.2-7.4 (m, 3H), 10.79 (s, 1H).

Referential Example 54: Synthesis of compound 123 {1-[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]piperidin-4-ylmethanol}

Ethyl 1-[8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-10,11-dihydro-5H-dibenz[b,f]azepin-2-ylmethyl]piperidine-4-carboxylate (0.61 g, 1.08 mmol) prepared in the step 1 of Referential Example 52 was dissolved in dichloromethane (10 mL), cooled at -78°C and stirred. A 1 mol/L solution of diisopropylaluminum hydride in toluene (3.20 mL, 3.20 mmol) was added to the reaction solution at the same temperature and the mixture was stirred for 3 hours at the same temperature and, after that, at room temperature for 10 minutes. A saturated aqueous solution of Rochelle salt and ethyl acetate were added to the reaction solution and the mixture was stirred for 30 minutes. The aqueous layer was extracted with ethyl acetate, the organic layer was dried over anhydrous magnesium sulfate and the solvent was evaporated *in vacuo*. The residue

was subjected to recrystallization from ethyl acetate to prepare compound 123 (0.26 g, 0.50 mmol, yield: 46%).

APCI-MS: m/z 524 ($[M + H]^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.0-1.15 (m, 2H), 1.23 (t, J = 7.5 Hz, 3H), 1.25-1.3 (m, 1H), 1.5-1.65 (m, 2H), 1.7-1.9 (m, 2H), 2.49 (s, 3H), 2.50 (s, 3H), 2.75 (q, J = 7.5 Hz, 2H), 2.95-3.05 (m, 4H), 3.15-3.25 (m, 5H), 3.25-3.50 (m, 4H), 5.32 (s, 2H), 6.81 (dd, J = 2.0, 8.5 Hz, 1H), 6.90-7.05 (m, 6H).

Referential Example 55: Synthesis of compound 124 [2-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-8-(2H-tetrazol-5-yl)-10,11-dihydro-5H-dibenz[b,f]azepine]

The operation similar to that in the step 1 of Referential Example 52 was conducted using compound 103 prepared in Referential Example 34 to prepare 8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-10,11-dihydro-5H-dibenz[b,f]azepine-2-carbonitrile in a yield of 83%.

The operation similar to that in the latter part of Referential Example 20 was conducted using the above compound to prepare compound 124 in a yield of 20%.

APCI-MS: m/z 465 ($[M + H]^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.24 (t, J = 7.7 Hz, 3H), 2.50 (s, 3H), 2.51 (s, 3H), 2.80 (q, J = 7.7 Hz, 2H), 3.0-3.1 (m, 2H), 3.3-3.35 (m, 2H), 3.40 (s, 3H), 5.38 (s, 2H), 6.90 (dd,

J = 2.2, 8.4 Hz, 1H), 6.95 (s, 1H), 7.02 (d, J = 2.2 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 2.2 Hz, 1H), 7.79 (dd, J = 2.2, 8.4 Hz, 1H).

Referential Example 56: Synthesis of compound 125 {[8-(2-Ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-10,11-dihydro-5H-dibenz[b,f]azepin-2-yl]acetic acid}

The operation similar to that in step 1 of Referential Example 52 was conducted using compound 105 prepared in Referential Example 36 to prepare [8-(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridin-3-ylmethyl)-5-methyl-10,11-dihydro-5H-dibenz[b,f]azepin-2-yl]acetonitrile in a yield of 94%.

The operation similar to that in Referential Example 38 was conducted using the above compound to give compound 125 in a yield of 86%.

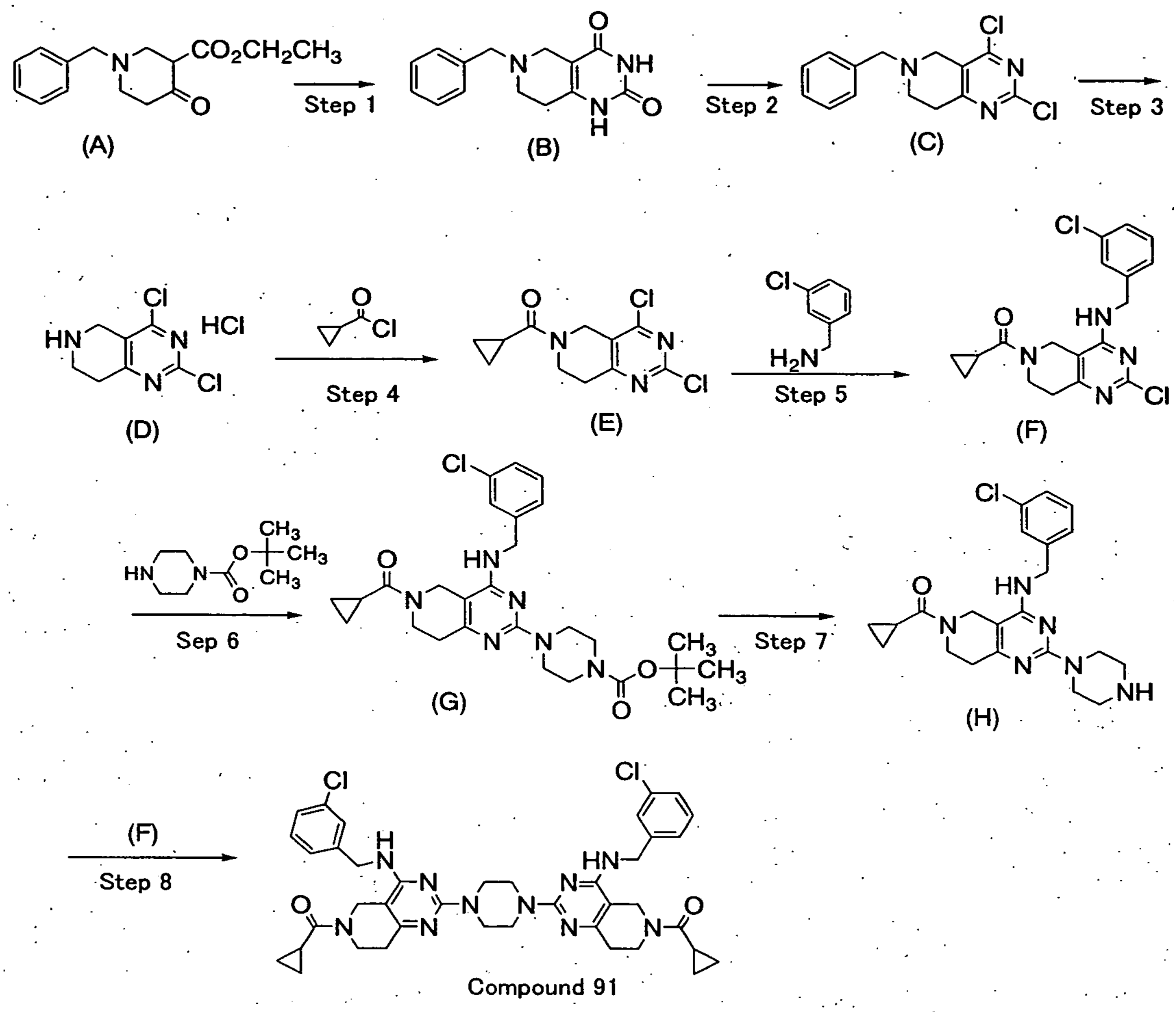
APCI-MS: m/z 455 ($[M + H]^+$)

^{1}H NMR (DMSO-d_6) δ(ppm): 1.22 (t, J = 7.3 Hz, 3H), 2.49 (s, 3H), 2.50 (s, 3H), 2.75 (q, J = 7.3 Hz, 2H), 2.9-3.1 (m, 4H), 3.19 (s, 3H), 3.42 (s, 2H), 5.32 (s, 2H), 6.81 (d, J = 8.1 Hz, 1H), 6.9-7.05 (m, 6H).

Referential Example 57: Synthesis of compound 91 {1,4-Bis[4-(3-chlorobenzylamino)-6-cyclopropylcarbonyl-7,8-dihydro-5H-pyrido[4,3-d]pyrimidin-2-yl]piperazine}

Compound 91 was synthesized according to the following

step 1 to step 8.



Step 1:

A commercially available compound (A) (100 g, 0.335 mol) was dissolved in ethanol (1,500 mL), then urea (100 g, 1.67 mol) and sodium methoxide (227 g, 1.18 mol) were added thereto and the mixture was made to react for 24 hours under the condition of heating to reflux. Progress of the reaction was confirmed by a thin-layer chromatography and, after cooled, crystals

separated out therefrom were filtered. The crystals were suspended in water, hydrochloric acid (6 mol/L) was added thereto and pH was adjusted to 6.0. Stirring was further conducted at room temperature for 1 hour and the crystals separated out therefrom were filtered and dried *in vacuo* to prepare compound (B) (60 g, yield: 70%).

Step 2:

Phosphorus oxychloride (300 mL) was added to compound (B) (30.0 g, 0.116 mol) prepared in the step 1 and the mixture was stirred under a heating condition for 5 hours. After confirming the progress of the reaction by a thin-layer chromatography, an excessive phosphorus oxychloride was evaporated *in vacuo*. After that, 2-propanol (300 mL) was added to the residue and a suspension containing the crystals separated out therefrom was stirred under the condition of heating to reflux for 1 hour and further stirred at room temperature for 1 hour. The crystals separated out were filtered and dried *in vacuo* to prepare compound (C) (33 g, yield: 85%).

Step 3:

Compound (C) (35.0 g, 0.106 mol) prepared in the step 2 was dissolved in 1,2-dichloroethane (850 mL), then triethylamine (14.9 mL, 0.107 mol) and 1-chloroethyl chloroformate (34.1 mL, 0.316 mol) were added thereto and the mixture was stirred under a condition of heating to reflux for 5 hours. After confirming the progress of the reaction by a

thin-layer chromatography, the reaction mixture was cooled, washed with water and a saturated aqueous saline solution successively and dried over anhydrous magnesium sulfate. The resulting solution was concentrated and the residue was purified by a column chromatography (silica gel, n-hexane:ethyl acetate = 3:1). The product was dissolved in methanol (850 mL) and stirred under a condition of heating to reflux for 1 hour. After confirming the progress of the reaction by a thin-layer chromatography, it was concentrated to dryness to prepare compound (D) (23.5 g, yield: 95%).

Step 4:

Compound (D) (11.8 g, 49.1 mmol) prepared in Step 3 was dissolved in dichloromethane (300 mL), then cyclopropanecarbonyl chloride (5.4 mL, 1.2 equivalents) and triethylamine (20.4 mL, 3.0 equivalents) were added thereto and the mixture was stirred at room temperature for 1 hour. The resulting reaction solution was washed with water and a saturated sodium bicarbonate solution and dried over magnesium sulfate. After evaporating the solvent, diisopropyl ether was added to the residue and the suspension was stirred for not shorter than 1 hour. After that, the crystals separated out therefrom were filtered and dried *in vacuo* to prepare compound (E) (12.5 g, yield: 94%).

Step 5:

Compound (E) (12.5 g, 45.9 mmol) prepared in the step

4 was dissolved in tetrahydrofuran (400 mL), then triethylamine (19.2 ml, 3 equivalents) and 3-chlorobenzylamine (11.2 mL, 2 equivalents) were added and the mixture was stirred at 40°C for 20 hours. The salt separated out therefrom was removed by filtration and the solvent was evaporated. The residue was purified by a chromatography (chloroform:methanol = 100:1 → 40:1) and a mixed solvent of hexane/ethyl acetate (3:1) was added to a concentrated residue of a fraction containing the objective substance so that the crystals were separated out. The suspension containing the crystals was stirred for 1 hour and the crystals separated out therefrom were filtered and dried *in vacuo* to prepare compound (F) (11.9 g, yield: 69%).

Step 6:

Compound (F) (5.0 g, 13.3 mmol) prepared in the step 5 was dissolved in dioxane (100 mL), then tert-butyl 1-piperazinecarboxylate (4.9 g, 2 equivalents) and sodium carbonate (14.0 g, 10 equivalents) were added thereto and the mixture was stirred at 90°C for 3 days. The resulting reaction solution was filtered to remove sodium carbonate, extraction was conducted by addition of water and chloroform to the filtrate and the organic layer was dried over magnesium sulfate. After evaporation of the solvent, a mixed solvent of hexane/ethyl acetate (3:1) was added and the suspension was stirred for 1 hour. After that, the crystals separated out therefrom were filtered and dried *in vacuo* to prepare compound (G) (6.4 g,

yield: 92%).

Step 7

A 20% solution of trifluoroacetic acid in dichloromethane (50 mL) was added to compound (G) (6.3 g, 12.0 mmol) prepared in the step 6 and the mixture was stirred at room temperature for 1 hour. After evaporating the solvent from the reaction solution, diisopropyl ether was added to the residue and the resulting suspension was stirred for 1 hour. After that, the crystals separated out therefrom were filtered and dried *in vacuo* to prepare compound (H) (4.9 g, yield: 97%).

Step 8:

Compound (H) (3.8 g, 8.90 mmol) prepared in the step 7 and compound (F) (4.5 g, 1.05 equivalent) prepared in the step 5 were dissolved in dioxane (100 mL), sodium carbonate (10.6 g, 10 equivalents) was added and the mixture was stirred at 90°C for 1 week. The resulting reaction solution was filtered to remove sodium carbonate, water was added to the filtrate and extraction was conducted with chloroform. The organic layer was dried over magnesium sulfate, the solvent was evaporated and the residue was purified by a column chromatography (ethyl acetate:triethylamine = 10:1). A mixed solvent of hexane/ethyl acetate (3:1) was added to a concentrated residue of a fraction containing the obejective substance and the resulting suspension was stirred for 1 hour. After that, the crystals separated out therefrom were filtered and dried *in vacuo* to

prepare compound 91 (1.0 g, yield: 23%).

APCI-MS: m/z 767 ($[M + H]^+$)

^1H NMR ($CDCl_3$) δ(ppm): 0.7-0.9 (m, 4H), 1.0-1.1 (m, 4H), 1.7-1.9 (m, 2H), 2.6-2.8 (m, 4H), 3.75 (s, 8H), 3.8-4.0 (m, 4H), 4.3-4.4 (m, 4H), 4.6-4.7 (m, 4H), 4.8-4.9 (m, 2H), 7.1-7.3 (m, 8H).

Referential Example 58: Construction of a host-vector system

(1) Construction of Gal4-ER expression plasmid pGERbsrR2

pSV2bsr (manufactured by Kaken Seiyaku) was cleaved with PvuII and EcoRI and subjected to a Klenow treatment to prepare a PvuII (blunt end)-EcoRI (blunt end) fragment of 2.6 kb.

ERαAF2 in pM containing the Gal4-ER chimeric gene [*Cell*, 54, 199 (1988); *Proc. Natl. Acad. Sci., USA*, 90, 1657 (1993)] (apportioned from Dr. Shigeaki Kato, University of Tokyo) was cleaved with AatII and NdeI and subjected to a Klenow treatment to prepare an AatII (blunt end)-NdeI (blunt end) fragment.

The above mentioned PvuII (blunt end)-EcoRI (blunt end) fragment derived from pSV2bsr and the AatII (blunt end)-NdeI (blunt end) fragment derived from ERαAF2 in pM were ligated to construct a plasmid pGERbsrR2. pGERbsrR2 is able to express a chimeric protein (Gal4-ER) consisting of a DNA binding domain of transcription factor Gal4p derived from a yeast (*Saccharomyces cerevisiae*) and a ligand binding domain of estrogen receptor.

(2) Construction of an inducible expression plasmid of firefly luciferase

pcDNA3 (Invitrogen) was cleaved with XhoI and subjected to a Klenow treatment to prepare a XhoI (blunt end) fragment. The fragment was ligated to construct pcDNA3 where cleaved sites by XhoI disappeared. pcDNA3 where the cleaved site with XhoI disappeared was cleaved with KpnI and subjected to a Klenow treatment to prepare a KpnI (blunt end) fragment. The fragment was ligated to construct pcDNA3 where cleaved sites with XhoI and KpnI disappeared. The plasmid was cleaved with BglII and subjected to a Klenow treatment to a prepare BglII (blunt end) fragment.

pAMoERC3Sc (Japanese Published Unexamined Patent Application No. 336,963/1993) was cleaved with XhoI and NsiI and subjected to a Klenow treatment to obtain a XhoI (blunt end)-NsiI (blunt end) fragment of 2.2 kb having an oriP sequence.

The above-mentioned BglII (blunt end) fragment derived from pcDNA3 where XhoI-cleaved site and KpnI-cleaved site disappeared and the XhoI (blunt end)-NsiI (blunt end) fragment derived from pAMoERC3Sc were ligated to construct a plasmid pcDNA3-oriP. pcDNA3-oriP was cleaved with XhoI and HindIII to obtain a XhoI-HindIII fragment.

pSE0luc2 (WO 98/14474) was cleaved with XhoI and NcoI and subjected to a Klenow treatment to obtain a XhoI (blunt end)-NcoI (blunt end) fragment comprising the

ampicillin-resistant gene. The fragments were ligated to construct a plasmid pASd1-luc1. After pASd1-luc1 was cleaved with XhoI and HindIII, a XhoI-HindIII fragment of 0.11 kb were obtained.

The above-mentioned XhoI-HindIII fragment derived from pcDNA3-oriP and the XhoI-HindIII fragment derived from pASd1-luc1 were ligated to construct a plasmid pcDNA3-oriP-Sd1. pcDNA3-oriP-Sd1 was cleaved with XhoI and KpnI to obtain a XhoI-KpnI fragment.

Four kinds of DNAs having nucleotide sequences represented by SEQ ID NOS: 1, 2, 3 and 4, respectively were synthesized by DNA synthetizer. When the synthetic DNAs were mixed and annealed, a double-stranded DNA having polyadenylation signal was constructed. Each of the synthetic DNAs was phosphorylated using T4 polynucleotide kinase, mixed and annealed to give a double-stranded DNA.

When the double-stranded DNA was ligated to the XhoI-KpnI fragment derived from pcDNA3-oriP-Sd1, a plasmid pcDNA3-oriP-Sd1-pA was constructed. pcDNA3-oriP-Sd1-pA was cleaved with XhoI and subjected to a Klenow treatment to obtain a XhoI (blunt end) fragment.

pFR-luc (manufactured by Stratagene) was cleaved with HindIII and BamHI and subjected to a Klenow treatment to obtain a HindIII (blunt end)-BamHI (blunt end) fragment of 0.14 kb.

The above-mentioned XhoI (blunt end) fragment derived

from pcDNA3-oriP-Sd1-pA and the HindIII-BamHI fragment derived from pFR-luc were ligated to obtain a plasmid pAGalSd1. The pAGalSd1 comprises a promoter having a sequence where Gal4p-responsive elements (UASG) are repeated for 5 times. pAGalSd1 was cleaved with EcoRI and subjected to a Klenow treatment to obtain a EcoRI (blunt end) fragment.

pSE0luc2 (WO 98/14474) was cleaved with HindIII and SacI and subjected to a Klenow treatment to prepare a HindIII (blunt end)-SacI (blunt end) fragment of 1.7 kb comprising the firefly luciferase gene.

The above-mentioned HindIII (blunt end)-SacI (blunt end) fragments derived from pSE0luc2 and the EcoRI (blunt end) derived from pAGalSd1 were ligated to construct a plasmid pAGalSd1-luc.

Among the two HindIII sites existing in pAGalSd1-luc, only a HindIII site far from the firefly luciferase gene was made disappeared by a Klenow treatment to construct pAGalSd4-luc.

pAGalSd4-luc was cleaved with Asp718 and subjected to a partial digestion with StuI to obtain an Asp718-StuI fragment of 9.5 kb derived from pAGalSd4-luc. The DNA fragments were subjected to a Klenow treatment and self-ligated to construct a plasmid pAGal9-luc.

(3) Construction of inducible expression vectors pAGal9-d and pAGal9-nd

Expression plasmid pAGal9-luc having oriP of Epstein-Barr

virus was cleaved with HindIII and SacI to prepare a HindIII-SacI fragment of 6.9 kb containing oriP.

pAMo-d (Japanese Published Unexamined Patent Application No. 211,885/2001) was cleaved with HindIII and SacI to prepare a HindIII-SacI fragment comprising the tetracycline-resistant gene (Tc^R).

The above-mentioned HindIII-SacI fragment derived from pAGal9-luc and the HindIII-SacI fragment derived from pAMo-d were ligated to construct a plasmid pAGal9-d where the firefly luciferase gene in pAGal9-luc was substituted with a stuffer sequence of pAMo-d. pAGal9-luc was cleaved with HindIII and SacI to prepare a HindIII-SacI fragment of 6.9 kb.

pAMo-nd (Japanese Published Unexamined Patent Application No. 211,885/2001) was cleaved with HindIII and SacI to prepare a HindIII-SacI fragment comprising the tetracycline-resistant gene.

The above-mentioned HindIII-SacI fragment derived from pAGal9-luc and the HindIII-SacI fragment derived from pAMo-nd were ligated to construct a plasmid pAGal9-nd where the firefly luciferase gene in pAGal9-luc was substituted with a stuffer sequence of pAMo-nd.

(4) Preparation of a cell line KJMGER8 where Gal4-ER expression plasmid pGERbsrR2 was integrated in chromosomal DNA of Namalwa KJM-1 cells

Gal4-ER chimeric transcription factor expression plasmid

pGERbsrR2 was dissolved in a TE buffer [10 mmol/L Tris-HCl (pH 8.0) and 1 mmol/L of ethylenediamine tetraacetate] so as to make 1 μg/μL and, after that, the plasmid, 4 μg for 6×10^6 cells, was transfected to Namalwa KJM-1 cells [*Cytotechnology*, 1, 151 (1988)] by an electroporation method [*Cytotechnology*, 3, 133 (1990)] to prepare transformed cells. Namalwa KJM-1 cell is a B-cell line adapted for serum-free culture, and capable of expressing the EBNA-1 gene.

The transformant was suspended in 8 ml of an RPMI 1640-ITPSG medium [a medium where a 1/40 amount of 7.5% $NaHCO_3$, 3% 200 mmol/L of L-glutamine solution (manufactured by Invitrogen), 0.5% penicillin-streptomycin solution (manufactured by Invitrogen comprising 5,000 units/ml of penicillin and 5,000 μg/ml of streptomycin), 10 mmol/L of N-2-hydroxyethylpiperazine-N'-2-hydroxypropane-3-sulfonic acid(HEPES), 3 μg/ml insulin, 5 μg/ml transferrin, 5 mmol/L sodium pyruvate, 125 nmol/L sodium selenite and 1 mg/ml galactose were added to an RPMI 1640 medium (manufactured by Nissui Seiyaku)] and cultured at 37°C in a CO_2 incubator for 24 hours.

After the cultivation, blasticidin S (KK-400: manufactured by Kaken Seiyaku) was added so as to make 2.0 μg/ml, dispensed in a 96-well plate (500 to 2,000 cells/well) and cultivation was carried out to obtain many stable transformants (single clones) where pGERbsrR2 was integrated in chromosomal DNA. Each transformant was subcultured in RPMI 1640-ITPSG

medium containing 2.0 μg/ml of blasticidin S.

By the method as mentioned below, an excellent stable transformant KJMGER8 cell having high induction ratio and low background upon non-inducing stage was selected from the above-mentioned stable transformants.

An inducible expression plasmid pAGalSd1-luc of firefly luciferase was transfected to each transformant by an electroporation method and cultured for 2 days.

After the cultivation, 17β-estradiol (E8875: manufactured by Sigma) (final concentration 10 nmol/L) was added and, after the cultivation for 24 hours more, the firefly luciferase activity was measured. For the measurement of the activity, a luminometer-LB 953 (manufactured by Berthold) was used, 100 μl of a buffer for dissolving the cells [1% Triton X-100, 100 mmol/L KH_2PO_4 (pH 7.8) and 1 mmol/L dithiothreitol] was automatically injected into the above culture solution, then 300 μl of a substrate solution [25 mmol/L glycylglycine (pH 7.8), 15 mmol/L $MgSO_4$, 5 mmol/L ATP and 0.33 mmol/L luciferin] was automatically injected and the amounts of emission of light during 10 seconds was measured for adopting as a luciferase activity. For comparison, luciferase activity under the condition where no 17β-estradiol was added was also measured.

Luciferase activity under the condition where 17β-estradiol was added and luciferase activity under the condition where no 17β-estradiol was added were compared,

induction ratio for gene expression was calculated, and KJMGER8 cell was selected as a clone with high induction ratio and low luciferase activity in the condition without addition of 17β-estradiol.

Referential Example 59: Construction of a reporter plasmid pACREpluc where firefly luciferase is a reporter

pACREpluc which is a reporter plasmid capable of expressing a firefly luciferase gene under the control of cAMP-responding element (CRE) was constructed by the following method. pACREpluc has oriP of Epstein Barr virus and the hygromycin-resistant gene.

pAMo [*J. Biol. Chem.*, 268, 22782 (1993); another name: pAMoPRC3Sc (Japanese Published Unexamined Patent Application No. 336,963/1993)] was partially digested with ClaI to obtain a DNA fragment where one site was cleaved. The DNA fragment was partially digested by MluI to obtain a ClaI-MluI fragment of 9.5 kb. pAGE248 [*J. Biol. Chem.*, 269, 14730 (1994)] was cleaved with ClaI and MluI to obtain a ClaI-MluI fragment of 1.5 kb comprising the hygromycin-resistant gene. The ClaI-MluI fragment derived from pAMo and the ClaI-MluI fragment derived from pAGE248 were ligated to construct a plasmid pAMoh.

pAMoh was cleaved with XhoI and HindIII to obtain a XhoI-HindIII fragment comprising the hygromycin-resistant gene. pAGal9-luc was cleaved with SalI and HindIII to obtain a SalI-HindIII fragment comprising oriP and Gal4UAS. The SalI-HindIII

fragment derived from pAGal9-luc and the above-mentioned XhoI-HindIII fragment derived from pAMoh were ligated to construct a plasmid pAGal9h.

pBluescriptII KS+ (manufactured by Toyoboseki) was cleaved with SalI and XhoI and subjected to a dephosphorylation using phosphatase (Alkaline Phosphatase E. coli C75; manufactured by Takara Shuzo) to obtain a SalI-XhoI fragment comprising the ampicillin-resistant gene. As a result of annealing of synthetic oligonucleotides having nucleotide sequences of SEQ ID NOs: 5 and 6, respectively, a double-stranded DNA containing two CRE sequences was prepared. The double-stranded DNA was ligated to the above SalI-XhoI fragment derived from pBluescriptII KS+ to construct a plasmid pBS-CREI comprising two CRE sequences. The pBS-CREI is a plasmid where the double-stranded DNA is inserted in such a direction that cleaved site with SalI and cleaved site with XhoI are regenerated and has each one of the above cleaved sites.

pBS-CREI was cleaved with ScaI and XhoI to prepare a ScaI-XhoI fragment comprising ori of a phage f1. pBS-CREI was cleaved with ScaI and SalI to prepare a ScaI-SalI fragment comprising ColE1 ori. The ScaI-XhoI fragment and the ScaI-SalI fragment derived from pBS-CREI were ligated to construct pBS-CREII comprising 4 CRE sequences.

pBS-CREII was cleaved with ScaI and XhoI to obtain a ScaI-XhoI fragment comprising ori of a phage f1. pBS-CREII

was cleaved with ScaI and SalI to obtain a ScaI-SalI fragment comprising ColE1 ori. The ScaI-XhoI fragment and the ScaI-SalI fragment derived from pBS-CREII were ligated to construct pBS-CREIV comprising 8 CRE sequences.

pBS-CREIV was cleaved with ScaI and XhoI to obtain a ScaI-XhoI fragment comprising ori of a phage f1. pBS-CREIV was cleaved with ScaI and SalI to obtain a ScaI-SalI fragment comprising ColE1 ori. The ScaI-XhoI fragment and the ScaI-SalI fragment derived from pBS-CREIV were ligated to construct pBS-CREVIII comprising 16 CRE sequences.

pBS-CREVIII was cleaved with XhoI, subjected to a Klenow treatment, and further cleaved with HindIII to obtain a HindIII-XhoI (blunt end) fragment comprising 16 CREs. pAGalSd1 was cleaved with MluI and HindIII to obtain a MluI-HindIII fragment of 1.4 kb. pAGal19h was cleaved with XbaI, subjected to a Klenow treatment, and further cleaved with MluI to give a XbaI (blunt end)-MluI fragment. The HindIII-XhoI (blunt end) fragment derived from pBS-CREVIII, the MluI-HindIII fragment derived from pAGalSd1 and the XbaI (blunt end)-MluI fragment derived from pAGal19h were ligated to prepare a plasmid pACREh.

pAGal9-luc was cleaved with XhoI and NotI to obtain a XhoI-NotI fragment comprising the firefly luciferase gene. pACREh was cleaved with XhoI and NotI to obtain a XhoI-NotI fragment comprising CRE sequences. The XhoI-NotI fragment derived from pAGal9-luc and the XhoI-NotI fragment derived from

pACREh were ligated to construct a plasmid pACREluc.

pACREluc was cleaved with HindIII, subjected to a Klenow treatment, and further cleaved with XhoI to obtain a HindIII (blunt end)-XhoI fragment comprising CRE and a HindIII (blunt end)-XhoI fragment comprising the firefly luciferase gene, respectively. The above-mentioned two HindIII (blunt end)-XhoI fragments derived from pACREluc were ligated to construct a plasmid pACRElucH in which HindIII site in upstream of CRE sequence in pACREluc disappeared.

pGL3-Enhancer vector (manufactured by Promega) was cleaved with HindIII and HpaI to obtain a HindIII-HpaI fragment comprising the luc+ gene (an improved firefly luciferase gene). pACREluc H was cleaved with NotI, subjected to a Klenow treatment, and further cleaved with HindIII to obtain a HindIII-NotI (blunt end) fragment containing CRE. The HindIII-HpaI fragment derived from pGL3-Enhancer vector and the HindIII-NotI (blunt end) fragment derived from pACREluc H were ligated to construct a plasmid pACREpluc.

Referential Example 60: Construction of a plasmid with inducible expression of GPR4

1 μg of mRNA derived from human lung (manufactured by Clontech) was used and a single-stranded cDNA was synthesized using a SUPERSCRIPT First-Stranded Synthesis System for RT-PCR (manufactured by Gibco). GPR4 cDNA was prepared by PCR using a solution (5 μl) where said single-stranded cDNA was diluted

with water to an extent of 250-fold as a template and synthetic DNA having nucleotide sequences represented by SEQ ID NO: 7 and NO: 8 as a GPR4 gene-specific primer. Sequence of the GPR4 gene-specific primer was designed on the basis of sequence information of GPR4 gene (GenBank Accession No. U21051). With regard to an enzyme, PfuTurbo DNA Polymerase (manufactured by Stratagene) was used. With regard to a buffer for conducting the PCR, a buffer of 10-fold concentration attached to the enzyme was used. The PCR was conducted by treating at 95°C for 5 minutes and conducting 30 cycles of reaction each comprising at 94°C for 1 minute, at 60°C for 1 minute and at 72°C for 1 minute using a thermal cycler DNA engine (manufactured by MJ Research).

The amplified GPR4 cDNA fragment was cleaved with HindIII and NotI which cleave a sequence designed on a primer. Fragment containing GPR4 cDNA was recovered by an agarose gel electrophoretic method.

Said cleaved fragment was integrated between HindIII and NotI of plasmid pAGal9-nd whereupon a plasmid pAGal9-GPR4 with inducible expression of GPR4.

Nucleotide of sequences of 5'-end and 3'-end of said cDNA were determined using a primer (synthetic DNA having sequences represented by SEQ ID NO: 9 and NO: 10) which is specific to nucleotide sequence in pAGal9-nd. A synthetic DNA which is specific to the determined nucleotide sequence was prepared and, when it was used as a primer, a nucleotide sequence farther

ahead was determined. As a result of repeating said method, total nucleotide sequence of said cDNA was determined and it was confirmed to code for GPR4. For the determination of the nucleotide sequence, a DNA sequencer 377 of Perkin Elmer and a reaction kit (ABI Prism™ BigDye™ Terminator Cycle Sequencing Ready Reaction kit: Applied Biosystems) were used.

A nucleotide sequence of DNA fragment integrated into a plasmid was determined and it was confirmed to code for GPR4.

Referential Example 61: Construction of assay cells of GPR4

2 μg of plasmid pAGal9-GPR4 with inducible expression of GPR4 and 2 μg of reporter plasmid pACREpluc were co-transferred into 6×10^6 cell of KJMGER8 by the above-mentioned electroporation. The transformants were suspended in 8 ml of an RPMI 1640-ITPSG medium and cultured in a CO_2 incubator at 37°C for 24 hours. After the cultivation, blasticidin S (2.0 μg/ml), hygromycin B (300 μg/ml) and geneticin (500 μg/ml) were added and cultivation was carried out for 14 days more, and stable transformants (called GPR4 assay cells) were obtained. The transformants were subcultured in an RPMI 1640-ITPSG medium containing blasticidin S (2.0 μg/ml), hygromycin B (300 μg/ml) and geneticin (500 μg/ml).

Similarly, control plasmid pAGal9-nd (2μg) and reporter plasmid pAGREpluc (2 μg) were co-transferred into KJMGER8 and a stable transformant (called control cell) was obtained.

Referential Example 62: Cloning of DNA coding for human GPR4 homolog derived from mice

Based upon the nucleotide sequence information of human GPR4 gene [Accession (AC) No. U21051], search was conducted using a database of NCBI as an object. As a result, mouse genome sequence (AC 073784) and plural expression sequence tag (EST) sequences (BF 178464, AA 968193, AA 798732, AI 840893 and AI 851037) were selected as sequences having high homology. A nucleotide sequence of a gene constructed from said mouse genome sequence and EST is shown in SEQ ID NO: 14 and an amino acid sequence of a polypeptide encoded by said gene is shown in SEQ ID NO: 13. When said amino acid was compared with the amino acid sequence of human GPR4 using an analysis program [GENETYX WIN ver. 5.0 (manufactured by Software)], it is confirmed that an identity score is 92.7%.

Therefore, it is presumed that a polypeptide having the amino acid sequence represented by SEQ ID NO: 13 is a human GPR4 homolog of mouse (mouse GPR4).

Accordingly, DNA coding for mouse GPR4 can be prepared by a PCR where mouse cDNA library which is commercially available or can be prepared by a known method is used as a template and an oligonucleotide which is able to be designed and synthesized depending upon the nucleotide sequence represented by SEQ ID NO: 14 as a primer set.

Referential Example 63: Cloning of DNA coding for a human

GPR4 homolog derived from rats

Based upon the nucleotide sequence information of human GPR4 gene (AC No. U21051), search was conducted using a database of NCBI as an object. As a result, two rat genome sequences (AC 119447.2 and AC 096180.2) and plural rat EST sequences (BF 544182, AI 170948, AI 008858, AI 235374, AI 502871 and BQ 194515) were selected as sequences having high homology. Based upon those sequences and the nucleotide sequence information of mice shown by SEQ ID NO: 14, oligonucleotides having the nucleotide sequences represented by SEQ ID NO: 15 and SEQ ID NO: 16 were prepared.

Each 1.0 μmol/L of said oligonucleotides was used as a primer set and 2 μL of cDNA prepared from mRNA derived from rat lung was used as a template whereby, in order to make the concentration of each component which is mentioned below 200 μmol/L, 40 μL of a reaction solution containing 2.5 units of dNTP (dATP, dGTP, dCTP, dTTP), Tag Gold (manufactured by Perkin Elmer) and 1 × Taq Gold (Mg plus) buffer (Perkin Elmer) was prepared and then a PCR was conducted under the following condition.

Thus, a thermal cycler PTC-200 (manufactured by MJ Research) was used and heating was conducted at 95°C for 10 minutes, then 30 cycles of reaction each comprises 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute were conducted and, further, heating at 72°C for 5 minutes was conducted.

From the resulting reaction solution of the PCR, 5 μL was collected and, after confirming by agarose gel electrophoresis that about 1.1 kb of DNA fragments presumed to be DNA coding for GPR4 were amplified, DNA fragments were eluted and recovered using QIAEX II Gel Extraction Kit (manufactured by Qiagen) according to the manual attached to the kit.

The above-recovered DNA fragments (50 ng) and 50 ng of pT7Blue T-Vector (manufactured by Novagen) were ligated using DNA Ligation Kit ver. 2 (manufactured by Takara Shuzo) according to the manual attached to the kit to prepare a recombinant plasmid DNA. A plasmid pT7RG was prepared by a conventional method from a transformant obtained by a transformation of *Escherichia coli* JM109 strain using the resulting recombinant plasmid DNA. As a result of determination of the total nucleotide sequence of the plasmid pT7RG, the pT7RG contains about 1.1 kb of cDNA having the nucleotide sequence represented by SEQ ID NO: 18. An amino acid sequence of a polypeptide correspongding to DNA comprising the nucleotide sequence represented by SEQ ID NO: 18 is shown in SEQ ID NO: 17. When the amino acid sequence was compared with amino acid sequences of human and mouse GPR4 using an analysis program [GENETYX WIN ver. 5.0 (manufactured by Software)], it is confirmed that identity scores are 93.0% and 99.2%, respectively.

Consequently, it has been clarified that a polypeptide

having the amino acids represented by SEQ ID NO: 17 is a human GPR4 homolog of rat (rat GRP4).

Example 1: Tablet

A tablet comprising the following composition is prepared by a conventional method.

Formulation

Compound 1	20 mg
Lactose	143.4 mg
Potato starch	30 mg
Hydroxypropyl cellulose	6 mg
Magnesium stearate	0.6 mg
	200 mg

Example 2: Injection solution

Injection solution comprising the following composition is prepared by a conventional method.

Formulation

Compound 5	2 mg
Pure soybean oil	200 mg
Pure egg yolk lecithin	24 mg
Glycerol for injection	50 mg
Distilled water for injection	1.72 ml
	2.00 ml

Industrial Applicability

The present invention provides an agent for prevention and/or treatment of asthma comprising a substance capable of suppressing the function involved in signal transduction of GPR4 as an active ingredient; an agent for prevention and/or treatment of asthma which comprises a nitrogen-containing tricyclic compound or a quaternary ammonium salt thereof, or a pharmaceutically acceptable salt thereof as an active ingredient.

Free Text of Sequence Listing

SEQ ID NO: 1 - Illustration of artificial sequence: Synthetic DNA

SEQ ID NO: 2 - Illustration of artificial sequence: Synthetic DNA

SEQ ID NO: 3 - Illustration of artificial sequence: Synthetic DNA

SEQ ID NO: 4 - Illustration of artificial sequence: Synthetic DNA

SEQ ID NO: 5 - Illustration of artificial sequence: Synthetic DNA

SEQ ID NO: 6 - Illustration of artificial sequence: Synthetic DNA

SEQ ID NO: 7 - Illustration of artificial sequence: Synthetic DNA

SEQ ID NO: 8 - Illustration of artificial sequence:

Synthetic DNA

SEQ ID NO: 9 - Illustration of artificial sequence: Synthetic DNA

SEQ ID NO: 10 - Illustration of artificial sequence: Synthetic DNA

SEQ ID NO: 15 - Illustration of artificial sequence: Synthetic DNA

SEQ ID NO: 16 - Illustration of artificial sequence: Synthetic DNA